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MOISTURE MOVEMENT IN WOOD ABOVE THE FIBER-SATURATION POINT[1]

By LOUIS W. REES, *assistant forester, Minnesota Agricultural Experiment Station,* and STANLEY J. BUCKMAN, *head of research department, American Creosoting Co.*

INTRODUCTION

Free water normally is present in wood at the beginning of the air-seasoning process, and also when wood is kiln-dried from the green or in some cases from the partly air-seasoned condition. As long as the wood contains free water in the cell cavities the movement of moisture to the surface may take place in a number of different ways. It may move as free water, as bound liquid, as vapor, or as a result of the combined action of any or all of these three possible mechanisms.

Because of the elimination of the possibility of free water movement in wood below the fiber-saturation point, the interpretation of the results obtained under these conditions is somewhat simplified. Several studies have been made recently of moisture movement through wood in which no free water was present. Martley (*15*)[2] and Stillwell (*26*) have investigated the movement of moisture through wood under conditions producing a more or less constant rate of movement. This was done by maintaining a constant high relative humidity at one end of the block and a constant low relative humidity at the opposite end in conjunction with methods which served to limit the moisture flow to one structural direction of the block. The results obtained did not indicate definitely the mechanism of moisture movement. Martley's analysis of his data showed, however, that a more rapid rate of moisture movement was obtained in the radial direction of Scotch pine with increasing moisture content of the wood below the fiber-saturation point. Stillwell's results indicated that the rates of moisture movement in the longitudinal direction of oak and ash varied from about one and one-half to seven times those in the radial and tangential directions.

Pidgeon and Maass (*16*) have studied the movement of moisture into small blocks composed entirely of the sapwood or heartwood of white spruce. These blocks were dried by prolonged evacuation in the presence of phosphorous anhydride and allowed to regain moisture at a vapor pressure of 4.58 mm of mercury and a temperature of 23° C. When the moisture movement was confined to the longitudinal direction, it was found that the time to half saturation for the heartwood varied from about two and one-fourth to seven and one-half times that for the sapwood; while for radial movement in the heartwood of white spruce the time to half saturation was about two and one-half times that for the sapwood. Although direct comparisons were not

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[2] Italic numbers in parentheses refer to Literature Cited, p. 186.

possible because the moisture movement did not take place through the same thickness of wood, the results further indicated that the rate of moisture movement in the radial direction was at least 20 times that in the longitudinal direction for both the heartwood and sapwood of white spruce. Pidgeon and Maass concluded from these results that moisture moves in wood below the fiber-saturation point in the form of vapor. However, since Pidgeon and Maass subjected evacuated wood to a definite vapor pressure in the absence of air their results are comparable only to pressure-permeability data and do not necessarily represent the relative rates of moisture diffusion to be expected under the conditions normally employed in seasoning wood.

In a recent paper, Buckman and Rees (*1*) concluded that moisture moves in wood below the fiber-saturation point predominantly by means of a mechanism involving the movement of the moisture across the cell cavities in the form of vapor and through the walls in the form of bound water. Evidence to support this conclusion was found in the fact that a definite relationship existed between the time required to gain half-saturation moisture content and the specific gravity for both sapwood and heartwood of different kinds of wood. Within the limits of the range of the specific gravity of the woods studied, this relationship appeared to be linear. However, other unpublished data collected by these authors showed that a straight-line relationship does not hold throughout the entire range of the specific gravity of wood, but that the relationship takes the form of an exponential function. The authors pointed out and discussed the reasons why such a relationship, when considered in the light of other available information, supports the predominant operation of a dual mechanism consisting of vapor movement across the cell cavities and bound liquid movement through the cell walls, in contrast to movement predominantly either in the form of vapor or bound liquid alone.

From theoretical calculations, using the approximate diameter and length of a softwood tracheid, the approximate thickness of the cell wall, and the relative rates of movement in the radial and tangential directions as compared to the longitudinal direction, Buckman and Rees (*1*) found that the relative rates of moisture movement as a vapor across the cell cavities and as a bound liquid through the cell walls were of the approximate order of magnitude of 100 to 1.

Tuttle (*28*), Sherwood, and coworkers (*3*, *8*, *18*, *19*, *20*, *21*), and Luikov (*13*) have used the diffusion equation to calculate the rate of moisture movement through wood and other solids when these materials contained free water. A good agreement was found between the calculated and experimental results. However, as Hawley (*11*) has stated, this agreement does not establish that the mechanism of moisture movement in wood corresponds to the assumptions of simple diffusion. Hawley has pointed out that, on the contrary, free water would create no potential which would cause it to diffuse from a higher to a lower concentration. Hawley explains the evidence of free water movement during the drying of some woods on the basis of a quite different mechanism involving the capillary forces which are set up in the cell cavities and the openings through the pit membranes during the drying process.

Although considerable work has already been done on the movement of moisture through wood below the fiber-saturation point, this information is not necessarily directly applicable to the seasoning of

wood as it is done in actual practice, since green wood always contains free water which must be removed during the seasoning process. The purpose of the present paper is to present: (1) Data on the relative rates of moisture movement in the different structural directions of heartwood and sapwood of different woods containing a maximum amount of free water at the beginning of the drying process, and (2) a discussion of these data in terms of the predominant mechanism of moisture movement in wood above the fiber-saturation point.

MATERIALS

Six different kinds of hardwoods and one softwood were used in the experiments. These woods were red oak (*Quercus borealis* Michaux f.), northern bur oak (*Q. macrocarpa var. olivaeformis* (Michaux f.), Gray), bitternut hickory (*Hicoria cordiformis* (Wangenheim) Britton), basswood (*Tilia glabra* Ventenat), silver maple (*Acer saccharinum* Linnaeus), hackberry (*Celtis occidentalis* Linnaeus), and red pine (*Pinus resinosa* Aiton).

The blocks used in the experiments, 2.5 by 2.5 by 5.0 cm in size, were sawed from green logs. The history of the material from which the blocks were obtained was known from the time the trees were felled until the wood was used. The blocks were cut to conform to the three structural directions of the wood and whenever possible samples were secured from both the heartwood and the sapwood of the same log.

METHODS

Immediately after the blocks were prepared, they were slowly dried to equilibrium with atmospheric conditions in the laboratory and maintained under these conditions until used in the experiment. Four blocks then were selected representing each structural direction of each of the hardwoods, and five blocks were selected representing each structural direction of the one coniferous wood used in the study. Blocks representing both the heartwood and the sapwood were selected whenever available. In addition, a representative block was selected with each group for preliminary moisture determinations. The oven-dry weight of each group of four or five blocks then was calculated, assuming that the group contained the same moisture content as the selected block.

The blocks representing the sapwood and heartwood of each different kind of wood then were placed in a separate container filled with tap water and subjected alternately to a vacuum of about 70 cm of mercury and pressure of 3.5 kg per square centimeter for a period of about 3 weeks. The water on the specimens was changed frequently in order to prevent a possible development of fungi or bacteria in the water. After the blocks had been treated in this way for about 1 week, the green volume of each set of blocks was determined by the water-immersion method. From the data thus accumulated, the theoretical maximum amounts of moisture which the blocks could absorb was calculated by the formula

$$M = 100\left(\frac{1}{S} - \frac{1}{1.55}\right)$$

in which M is maximum percentage of moisture in the wood when all of the air space is completely filled with water, S is the specific gravity of the wood based on the calculated oven-dry weight and the volume

of the wood at a moisture content above the fiber-saturation point, and 1.55 is the density of wood substance. Soaking of the blocks in the manner described above was continued until they had reached approximately these calculated maximum moisture contents.

When the blocks were saturated, they were removed from the water, wiped with a damp cloth, and allowed to stand in the atmosphere of the laboratory for about 15 minutes in order to remove most of the surface moisture. They then were coated on five surfaces with 16 coats of vulcanized rubber latex. The end of each block, which was to be exposed later, was covered during the coating process with a sheet of tinfoil, in order to retard evaporation at the uncoated end. After coating, the blocks were returned to the water with the tinfoil

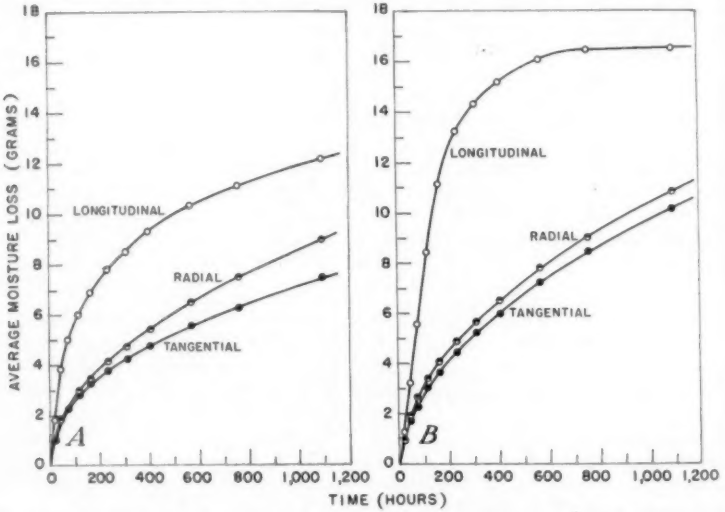

FIGURE 1.—Average moisture loss in grams of blocks of red oak heartwood (*A*) and red pine heartwood (*B*) when held at 30° C. and 81 percent humidity, the moisture movement having been confined to the three structural directions.

still attached and stored in this manner until the start of the experiment. At this time, the blocks were removed and allowed to stand in the laboratory for at least 2 hours in order that evaporation of water from the surface of the rubber coating could take place. The tinfoil then was removed and a rubber band placed around the block near the exposed end in order to prevent the coating from coming loose at this place. Each group of blocks was weighed to the nearest 0.01 and then placed in a cabinet maintained at a temperature of 30° C. and a relative humidity of 81 percent. At the end of 19.5 hours, the groups of blocks were weighed again and the decrease in weight was calculated. The weighings were repeated thereafter at what appeared to be suitable intervals of time. The entire series of blocks were run at the same time, thereby more or less eliminating the influence of any minor fluctuations in temperature and humidity on the relative nature of results obtained.

Immediately after the last weights were taken, the rubber coatings were removed and the blocks weighed, the weights of the coatings

being obtained by a subtraction of the results of these two determinations. The oven-dry weights were determined after the blocks had been dried to a constant weight at atmospheric pressure and a temperature of 105° C. From these data, the specific gravities based on the oven-dry weight and green volume were calculated. The dimensions of the surface exposed during the drying process were determined from the resoaked blocks after they had been oven-dried.

EXPERIMENTAL RESULTS

The data showing the average moisture loss in grams with time for the groups of blocks employed in investigating the moisture movement in the three structural directions of the different kinds of wood are given in table 1. Representative curves showing these data graphically are given in figure 1.

TABLE 1.—*Average total moisture loss in grams when the moisture movement was confined to the three structural directions of the different woods*

Kind of wood and direction	Average moisture loss [1] when the elapsed time was—										
	19.5 hours	44 hours	74 hours	116 hours	165 hours	236 hours	312 hours	408 hours	572 hours	764 hours	1,100 hours
Red oak heartwood:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Longitudinal	1.85	3.86	5.03	6.06	6.86	7.78	8.51	9.53	10.36	11.17	12.22
Radial	1.13	1.84	2.39	2.98	3.49	4.16	4.75	5.45	6.51	7.52	9.03
Tangential	1.08	1.75	2.24	2.81	3.27	3.79	4.24	4.74	5.53	6.29	7.46
Bur oak heartwood:											
Longitudinal	1.33	3.84	5.68	7.18	8.15	9.16	9.89	10.70	11.70	12.41	13.30
Radial	1.00	1.67	2.22	2.80	3.37	4.08	4.74	5.51	6.59	8.07	9.24
Tangential	.96	1.63	2.14	2.76	3.33	4.09	4.76	5.53	6.64	8.51	9.24
Hickory heartwood:											
Longitudinal	1.55	3.79	5.28	6.55	7.38	8.25	8.84	9.43	10.17	10.74	11.41
Radial	.92	1.61	2.11	2.66	3.17	3.84	4.37	4.98	5.95	6.83	8.13
Tangential	.82	1.42	1.78	2.28	2.66	3.17	3.60	4.12	4.84	5.54	6.55
Basswood heartwood:											
Longitudinal	1.55	4.21	6.77	10.93	14.92	18.45	19.57	20.49	21.08	21.29	21.35
Radial	1.49	3.99	6.15	8.22	9.50	10.89	12.03	13.19	14.78	16.19	18.24
Tengential	1.76	4.53	7.07	10.42	13.42	16.54	17.82	18.65	19.52	20.07	20.55
Silver maple heartwood:											
Longitudinal	1.48	3.96	6.53	10.30	13.04	15.43	16.69	17.41	18.12	18.50	18.73
Radial	1.10	2.28	3.11	4.33	4.88	5.94	6.86	7.93	9.52	11.10	13.21
Tangential	1.02	1.81	2.35	3.02	3.55	4.28	4.90	5.58	6.59	7.55	8.73
Silver maple sapwood:											
Longitudinal	1.29	3.76	6.08	9.70	11.70	13.62	14.81	15.66	16.45	16.97	17.17
Radial	1.55	3.87	5.44	7.21	8.57	10.20	11.55	12.90	14.45	15.52	16.44
Tengential	1.56	3.43	4.48	5.60	6.35	7.23	7.81	8.45	9.37	10.21	11.44
Hackberry heartwood:											
Longitudinal	2.29	3.45	5.37	7.84	9.40	10.68	11.50	12.25	13.04	13.70	14.33
Tangential	1.49	2.06	2.74	3.50	4.09	4.83	5.45	6.11	7.03	7.93	9.20
Hackberry sapwood:											
Longitudinal	1.46	3.61	5.83	8.62	10.89	12.79	14.07	15.25	16.44	17.23	17.86
Tangential	1.18	2.08	2.77	3.57	4.26	5.14	5.92	6.80	8.06	9.28	11.00
Red pine heartwood:											
Longitudinal	1.28	3.23	5.55	8.46	11.14	13.27	14.31	15.19	16.07	16.44	16.48
Radial	.94	1.87	2.60	3.41	4.07	4.90	5.64	6.48	7.74	9.01	10.80
Tangential	.92	1.68	2.29	3.03	3.65	4.45	5.18	5.98	7.20	8.40	10.12
Red pine sapwood:											
Longitudinal	1.83	3.55	5.52	8.06	10.49	13.38	15.52	16.65	17.28	17.50	17.52
Radial	1.67	3.98	5.80	7.67	9.17	10.84	12.29	13.82	15.79	16.91	17.69
Tangential	1.07	1.94	2.61	3.40	4.08	4.93	5.71	6.63	8.01	9.40	11.30

[1] Zero loss at zero hours.

After curves, similar to those shown in figure 1, had been prepared for all of the woods used in the experiments, the rates of moisture loss in grams per hour were determined for selected points on each curve from mechanically constructed lines having the same slope as the tangents at these points. These lines were constructed by erecting a perpendicular to the tangent for each selected point on the curve with

the aid of a plateglass mirror about 6 inches long and three-quarters of an inch high. One edge of the mirror was ground so that the reflecting surface would stand at right angles to the paper on which the curves were drawn. A line having the same slope as the tangent to the curve

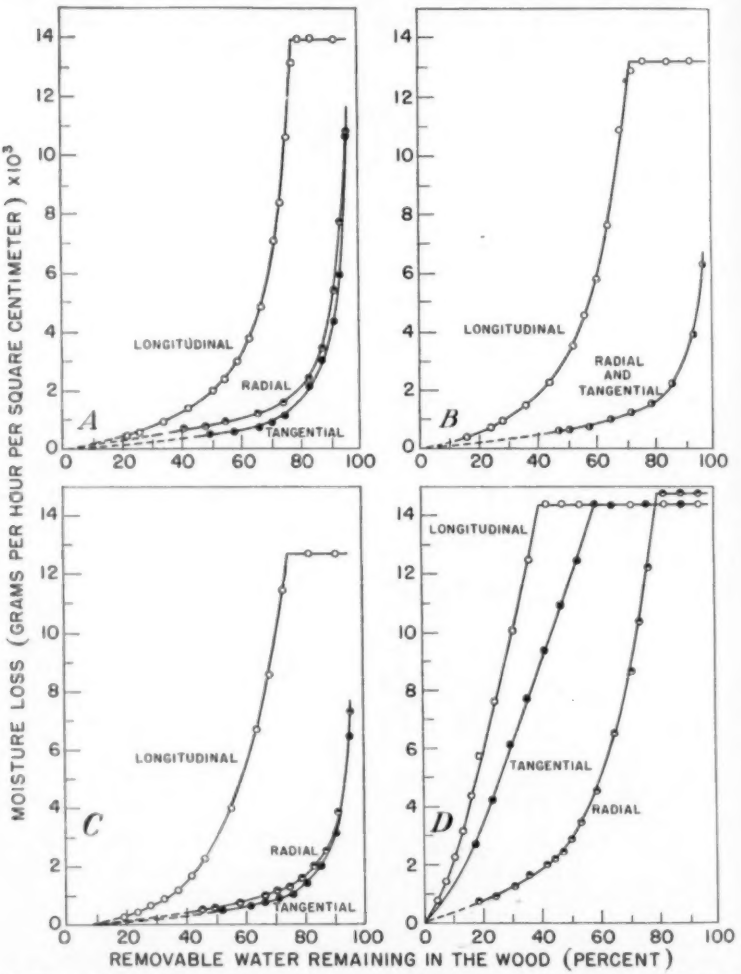

FIGURE 2.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) red oak heartwood; (*B*) bur oak heartwood; (*C*) hickory heartwood; (*D*) basswood heartwood.

at the point of intersection of the perpendicular and the curve then was constructed by drawing a line at right angles to this perpendicular.

The rates of moisture loss in grams per hour obtained by the above method were divided by the average green cross-sectional areas of the groups of blocks in order to determine the rates of moisture loss in

grams per hour per square centimeter of exposed surface. Shrinkage was not taken into account in calculating the rates of moisture loss when a portion of the blocks was below the fiber-saturation point. However, the magnitude of the difference in amount of shrinkage for

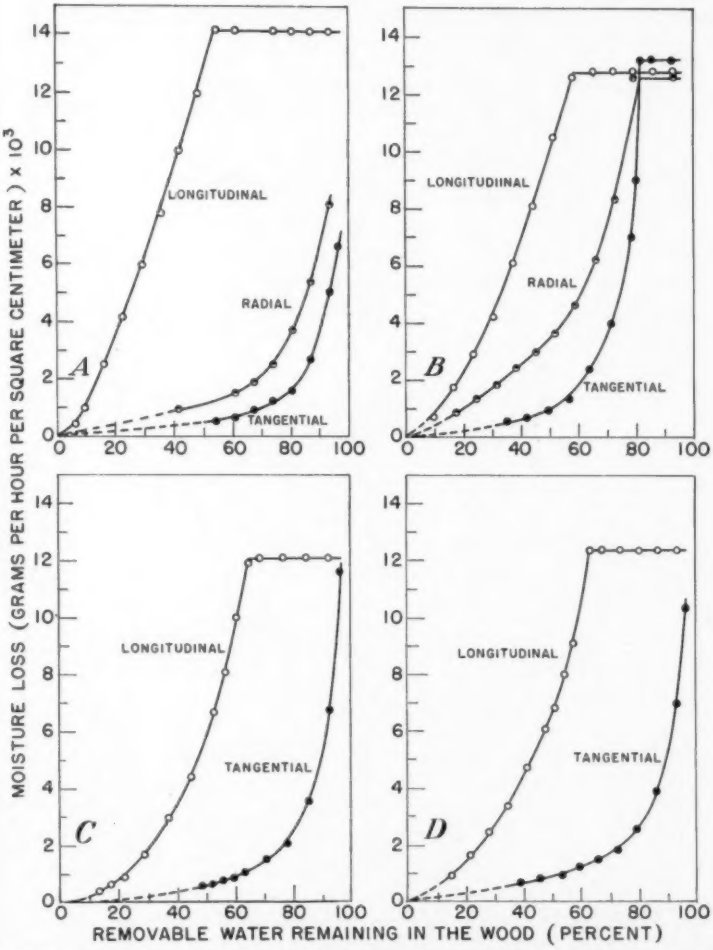

FIGURE 3.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) silver maple heartwood; (*B*) silver maple sapwood; (*C*) hackberry heartwood; (*D*) hackberry sapwood.

the different woods when dried to an equilibrium moisture content of about 16 percent, is comparatively small. The percentage of total removable water which still remained in the blocks then was calculated in the case of each point for which the rate of moisture loss was determined. The data obtained by the foregoing procedure are shown graphically in figures 2, 3, and 4.

From curves given in figures 2, 3, and 4, the following data were obtained: (1) The rates of moisture loss during the constant rate period, (2) the percentages of total removable water remaining in the wood at the critical point, which will be referred to in the subsequent discussion as "the critical moisture content," and (3) the rates of moisture loss when 50 and 25 percent of the total removable water still remained in the wood. These data, the specific gravity of the various woods, and the ratios between the rates of moisture loss for movement in the different structural directions of the different woods when 50 and 25 percent of the total removable water still remained in the wood, are given in table 2.

TABLE 2.—*Summary of the results for moisture movement in the 3 structural directions of the different kinds of wood*

Kind of wood	Specific gravity			Water loss per hour per square centimeter of exposed surface during the constant rate period			Percentage of total removable water in the wood at the critical moisture content			Water loss per hour per square centimeter of exposed surface when 50 percent of the removable water remained in the blocks		
	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential	Longitudinal	Radial	Tangential
				Grams	*Grams*	*Grams*	*Pct.*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*
Red oak heartwood	0.620	0.605	0.581	0.01399	(1)	(1)	77.5	(1)	(1)	0.00195	0.00083	0.00050
Bur oak heartwood	.575	.560	.590	.01320	(1)	(1)	72.0	(1)	(1)	.00341	.00063	.00063
Hickory heartwood	.632	.654	.643	.01270	(1)	(1)	75.0	(1)	(1)	.00288	.00058	.00043
Basswood heartwood	.367	.357	.375	.01440	0.01480	0.01440	40.0	80.0	59.0	.01440	.00286	.01170
Silver maple heartwood	.437	.448	.475	.01420	(1)	(1)	54.2	(1)	(1)	.01275	.00113	.00050
Silver maple sapwood	.493	.477	.468	.01280	.01260	.01320	58.7	81.5	81.5	.01000	.00348	.00099
Hackberry heartwood	.550	(2)	.528	.01210	(1)	(1)	64.8	(1)	(1)	.00577	(2)	.00057
Hackberry sapwood	.468	(2)	.474	.01237	(1)	(1)	63.3	(1)	(1)	.00667	(2)	.00086
Red pine heartwood	.361	.363	.374	.01173	(1)	(1)	48.0	(1)	(1)	.01173	.00090	.00068
Red pine sapwood	.412	.405	.416	.01110	.01045	(1)	50.0	70.0	(1)	.01110	.00442	.00112
Average				.01285	.01262	.01380						

Kind of wood	Comparative rates of moisture loss in the different structural directions, when 50 percent of the removable water remained in the block			Water loss per hour per square centimeter of exposed surface when 25 percent of the removable water remained in the blocks			Comparative rates of moisture loss in the different structural directions, when 25 percent of the removable water remained in the block		
	Longitudinal Radial	Longitudinal Tangential	Radial Tangential	Longitudinal	Radial	Tangential	Longitudinal Radial	Longitudinal Tangential	Radial Tangential
				Grams	*Grams*	*Grams*			
Red oak heartwood	2.3	3.9	1.7	0.00058	0.00038	0.00022	1.5	2.6	1.7
Bur oak heartwood	5.0	5.0	1.0	.00076	.00026	.00026	2.9	2.9	1.0
Hickory heartwood	5.0	6.7	1.3	.00050	.00022	.00017	2.3	2.9	1.3
Basswood heartwood	5.0	1.2	.2	.00760	.00099	.00466	7.7	1.6	.2
Silver maple heartwood	11.3	25.5	2.3	.00493	.00056	.00025	8.8	19.7	2.2
Silver maple sapwood	2.9	10.1	3.5	.00323	.00138	.00028	2.3	11.5	4.9
Hackberry heartwood	(2)	10.1	(2)	.00128	(2)	.00015	(2)	8.5	(2)
Hackberry sapwood	(2)	7.8	(2)	.00198	(2)	.00034	(2)	5.8	(2)
Red pine heartwood	13.0	17.2	1.3	.00378	.00037	.00027	10.2	14.0	1.4
Red pine sapwood	2.5	9.9	4.0	.00442	.00182	.00053	2.4	8.3	3.4
Average	5.9	9.7	1.9				4.8	7.8	2.0

[1] No constant rate period or critical moisture content observed.
[2] No samples in which the moisture movement was confined to the radial direction.

A curve showing the relationship between the specific gravity and the critical moisture content for moisture movement in the longitudinal direction of the different woods is given in figure 5. Figure 6, *A* and *B*, shows the relationship between specific gravity and the rates of moisture loss when 50 and 25 percent of the total removable water remained in the wood. The curves given in figures 5 and 6 were fitted to the data by the method of least squares. It was decided that a straight line would best fit the data given in figure 5. In order to determine the type of equation which would best express the data given in figure 6, the various points for the rates of moisture loss in the longitudinal direction when 50 percent of the total removable water still remained in the wood, were plotted on logarithmic and

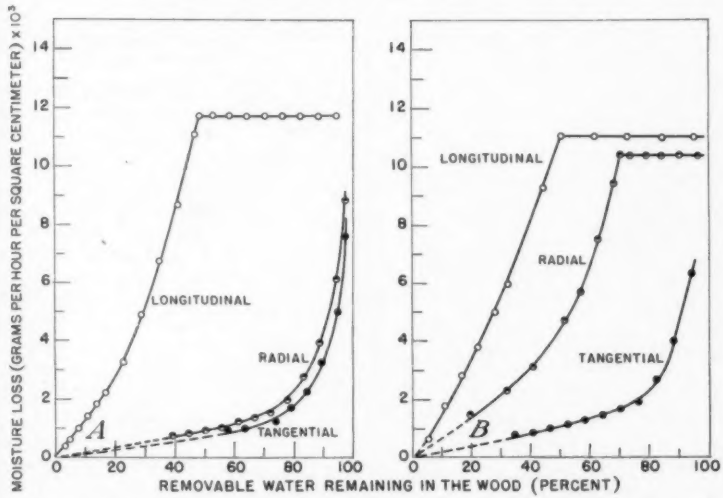

FIGURE 4.—Relation between the rate of moisture loss and the percentage of removable water remaining in the wood for the different structural directions of (*A*) red pine heartwood; (*B*) red pine sapwood.

semilogarithmic paper. A straight line was obtained on semilogarithmic paper, when the rate of moisture loss was expressed as the dependent variable and as a logarithmic function. The general equation which expresses such a relationship is

$$\log Y = a + bX$$

in which, Y is the rate of moisture loss, X is the specific gravity, a is a constant, and b is the slope of the straight line. It was assumed that the same general type equation would express the relationship between the rate of moisture loss and the specific gravity of the wood when the moisture movement was confined to the radial and tangential directions. This assumption also was used in deriving the equations for the curves showing the relationship between rate of moisture loss and specific gravity when 25 percent of the total removable water still remained in the wood.

DISCUSSION OF RESULTS

Sherwood (*18*), in some of his early papers on the drying of solids, discusses three possible mechanisms by which the water in a solid may diffuse to the surface and out into the surrounding atmosphere. These general mechanisms of drying (*18, p. 976*) are:

I.—Evaporation of the liquid at the solid surface; resistance to internal diffusion of liquid small as compared with the resistance to removal of vapor from the surface.

II.—Evaporation at the solid surface; resistance to internal diffusion of liquid great as compared with the resistance to removal of vapor from the surface.

III.—Evaporation in the interior of the solid; resistance to internal diffusion of the liquid great as compared with the total resistance to removal of vapor.

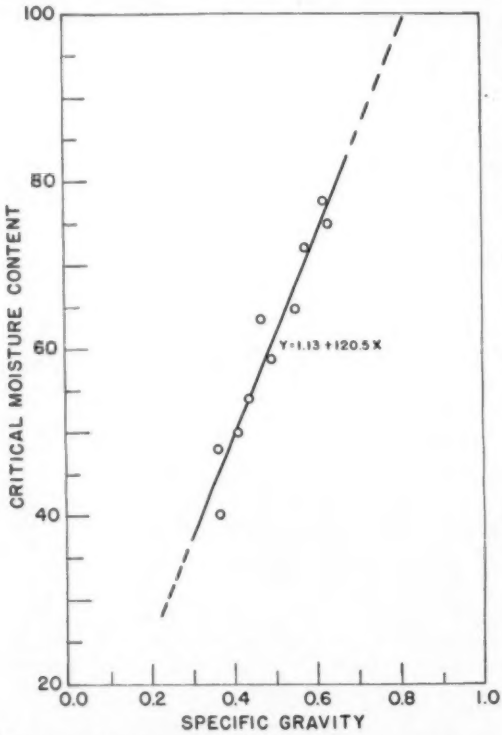

FIGURE 5.—Relation between the specific gravity and the critical moisture content of the different woods when the moisture movement was confined to the longitudinal direction.

Fisher (*5, 6*) in some of his early work on the drying of solids, concluded that the drying curves he obtained for wool, sand clay, and wood were divisible into three or four parts. Sherwood and coworkers (*3, 8, 18, 19, 20, 21*) and Luikov (*13*) have studied the drying of numerous solids from the standpoint of the foregoing theoretical considerations given by Sherwood (*18, pt. 2*) and have presented detailed mathematical analyses of the results obtained. They concluded that the drying curves for the solids used in their studies generally were divisible into three parts. Explanations were given in each case for the existence of the different portions of the drying curve. The materials investigated included wood, whiting slabs, soap slabs, compressed wood pulp, newsprint paper, brick clay, bagasse fiberboard, and peat.

Sherwood and coworkers and Luikov explain that, when the solid is very wet, the rate of moisture loss from the surface is similar to that from a free water surface; therefore, under constant drying conditions, the rate of drying remains constant. During this period, the controlling factor is the rate of evaporation through the surface air

film. As drying continues, a critical point eventually is reached on the drying curve where the rate of moisture loss begins to decrease, and the range from this point to the equilibrium moisture content is called the "falling rate period." As previously stated, the falling rate period was found to be divisible into two distinct zones called (1) the zone of unsaturated surface drying, and (2) the zone where internal liquid diffusion controls. The first of these zones follows immediately after the critical point has been reached. In this zone, it was concluded that there is a constant decrease in the rate of moisture loss with a decreasing percentage of the total removable water. The mechanism of evaporation was considered to be essentially the same as that during the constant rate period and the rate of moisture

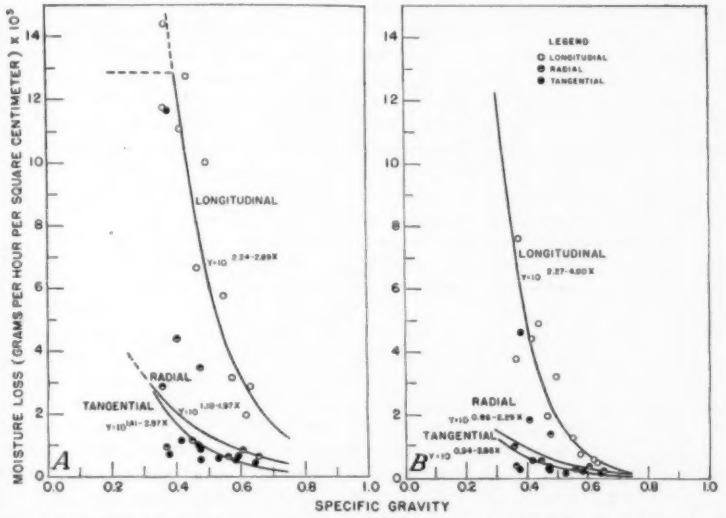

FIGURE 6.—Relation between the specific gravity and the rate of moisture loss when the moisture movement was confined to the three structural directions of the different woods and when the amount of removable water remaining in the blocks was 50 percent (*A*) and 25 percent (*B*).

loss was considered to be independent of the thickness of the material. Likewise in this zone, as during the constant rate period, the resistance to internal liquid diffusion was considered small in comparison to the resistance to vapor diffusion through the surface air film. As the rate of drying continued to decrease, a second critical point was found to exist beyond which resistance to the internal diffusion of the liquid controlled the rate of drying. During this period, it was concluded that the zone of evaporation retreated from the surface, and the water vapor was required to move through both the relatively dry surface of the solid and through the surface air film.

A study of the curves given in figures 2, 3, and 4 will show that these drying curves are divisible into a constant rate period and a falling rate period when the moisture movement was confined to the longitudinal direction of the various woods, and also when the moisture movement was confined to the radial and tangential directions

of basswood heartwood, silver maple sapwood, and to the radial direction of red pine sapwood. For the remainder of the woods the drying curves for moisture movement in the radial and tangential directions exhibited only the falling rate period. In all cases where a constant rate period is present there is evidence of an abrupt change to the falling rate period at the critical point. The results do not show, however, that the falling rate period is clearly divisible into two zones. The upper portion of the falling rate curve tends to approach a straight line, but there is no evidence of an abrupt change in the rate of moisture loss throughout this portion of the curve, thus eliminating suggestions of a second critical point. Except in the heavier woods, such as oak and hickory, most of the removable water was lost from the wood during the experiment and the drying curves approached zero, especially when the moisture movement was confined to the longitudinal direction. Considerable moisture was still present in the remainder of the blocks at the end of the experiment, but in all cases 50 percent or more of the removable water had been lost from the wood. It was noted during the latter part of the experimental run that the humidity in the cabinet had decreased slightly, thereby drying the woods at a somewhat faster rate. This had the effect of throwing the last points of the drying curve slightly out of line with the remainder, especially for those woods in which considerable moisture still was present.

A comparison of the rates of moisture loss for the different structural directions of the various woods, when 50 and 25 percent of the total removable water still remained in the wood, is given in table 2. When 50 percent of the total removable water remained in the woods, the rate of moisture loss in the longitudinal direction varied from 2.3 to 13.0 times that in the radial direction and from 1.2 to 25.5 times that in the tangential direction. The average rate of moisture loss in the longitudinal direction was 5.9 times that in the radial direction and 9.7 times that in the tangential direction. When 25 percent of the total removable water still remained in the woods, the rate of moisture loss for moisture movement in the longitudinal direction varied from 1.5 to 10.2 times that in the radial direction and from 1.6 to 19.7 times that in the tangential direction. The average rate of moisture loss, when the moisture movement was confined to the longitudinal direction, was 4.8 times the rate for movement in the radial direction and 7.8 times the rate for movement in the tangential direction. From the data in table 2, it also is evident that the rates of moisture loss for moisture movement in the radial direction varied from 0.2 to 4.0 times those for moisture movement in the tangential direction when 50 percent of the total removable water remained in the wood, and from 0.2 to 4.9 times those for movement in the tangential direction when 25 percent of the total removable water remained in the wood. The average rate of moisture loss for moisture movement in the radial direction varied from 1.9 times that in the tangential direction when 50 percent of the total removable water remained in the wood to 2.0 times that in the tangential direction when 25 percent of the removable water remained in the wood. It should be pointed out that in many cases the rates of moisture loss at the time when 25 percent of the removable water still remained in the wood were read from the extrapolated portion of the curves given

in figures 2, 3, and 4. These results show, however, that average ratios of nearly the same order of magnitude were maintained throughout the falling rate period.

The average ratios obtained for moisture movement in the different structural directions are of a similar order of magnitude to those previously reported by Buckman and Rees (*1*) for moisture movement in several coniferous woods below the fiber-saturation point. It was found that the time to attain half-saturation when the moisture movement was confined to the different structural directions was about five times as great in the radial and tangential direction as in the longitudinal direction.

The results given in table 2 show the following relative relationships between the rates of moisture loss from the heartwood and sapwood of silver maple, hackberry, and red pine when 50 percent of the total removable moisture remained in the wood. For moisture movement in the longitudinal direction of silver maple, the rate of moisture loss from the heartwood was approximately 1.3 times that for the sapwood. In the case of hackberry, the results were reversed, and the rate of moisture loss for movement in the longitudial direction of the sapwood was about 1.2 times that for the heartwood. The relative rates of moisture loss from the heartwood and sapwood of both of these woods were in the direction expected on the basis of the specific gravity relationship shown in figure 6. Both the heartwood and sapwood of red pine were still in the constant rate period when 50 percent of the removable water remained in the wood, and the variations in rate of moisture loss from the heartwood and sapwood were no greater than the normal variations for the different woods during this portion of the drying period.

Relationships comparable to the foregoing are shown by the rates of moisture loss in the heartwood and sapwood of silver maple and hackberry when 25 percent of the total removable water remained in the wood. In the red pine, however, the rate of moisture loss in the sapwood was about 1.2 times that in the heartwood, which is contrary to the more or less general relationship with specific gravity shown in figure 6.

Comparisons also can be made of the rates of moisture loss from the heartwood and sapwood when the moisture movement was confined to the radial and tangential directions. When 50 percent of the total removable water remained in the wood, the rates of moisture loss from the sapwood varied from 1.5 times that from the heartwood in the case of movement in the tangential direction of hackberry to 4.9 times that for the heartwood of red pine when the moisture movement was confined to the radial direction. When 25 percent of the removable water still remained in the wood, the rate of moisture loss from the sapwood of silver maple was 1.1 times that from the heartwood when movement was confined to the tangential direction. The rate of moisture loss in the heartwood of red pine was 4.9 times that from the sapwood when the moisture movement was confined to the radial direction. These results show that there was less resistance to moisture movement in the radial and tangential directions in the sapwood than in the heartwood.

These results do not appear to agree completely with those of Buckman and Rees (*1*), who found that the rates of moisture move-

ment below the fiber-saturation point were not significantly different in the sapwood and heartwood of Norway (red) pine and white spruce. However, in their study, comparisons could be made of the rates of moisture movement in the sapwood and heartwood of the two kinds of wood only when the moisture movement was confined to the longitudinal direction and when it was confined to the tangential direction of white spruce. As will be noted, the rates of moisture movement observed in the present study are probably not even significantly different in the heartwood and sapwood of red pine of the same specific gravities when the moisture movement was in the longitudinal direction. In addition, since these data were published, Erickson [3] has obtained information showing that there is a gradual decrease in the pressure permeability of white spruce in the longitudinal direction when samples are taken in order from the outer to the inner portions of the sapwood. Erickson, Schmitz, and Gortner (*4*) also have found that seasoning may have a significant effect on the permeability of white spruce sapwood, seasoning tending to decrease pressure permeability. This information indicates that sapwood samples selected on the basis of moisture content may tend to resemble heartwood from the standpoint of pressure permeability and also that seasoning may increase the pressure permeability of white spruce sapwood. Such data help, therefore, to explain the lack of complete agreement which may exist between the results of the two investigations insofar as these results should be correlated with the pressure permeability of the woods.

It is of considerable interest to review the data obtained in terms of the possible mechanisms of moisture movement in wood which is above the fiber-saturation point, at least at the beginning of the drying period. As previously noted, moisture may move to the surface under such conditions as free water, as bound liquid, as vapor, or by a combination of any or all of these three possible mechanisms. In all probability, all of the different mechanisms are in operation to some extent during the drying of wood above the fiber-saturation point. The primary question deals with the selection of the mechanism or combinations of such which predominate during the drying process. Certain of the pertinent factors influencing moisture movement by the different mechanisms from the standpoint of the discussion of the data given in this paper are as follows: (1) Free water movement would depend upon the size, number, and length of the capillaries between the cavities containing free water and the proportions of air and water in these cavities. Other conditions being equal, the rates of free water movement through the openings between the cavities would vary directly as the fourth powers of the radii of these capillaries. (2) Moisture movement as bound liquid would depend upon the amount of cell-wall substance along which movement can take place and the effectiveness of the cell-wall substance as a moisture-conducting medium. (3) Moisture movement in the form of vapor would depend upon the number and size of the capillary openings between the cells of the wood and the number and size of other openings that may contribute to the continuous effective open area available for vapor movement in the different structural directions, such as the presence of open vessels which furnish channels for vapor move-

[3] ERICKSON, H. D. PERMEABILITY OF WOODS TO DIFFERENT LIQUIDS AND FACTORS AFFECTING THE RATE OF FLOW. Master's thesis, Univ. of Minn. Library, Minneapolis, Minn. 1936.

ment in the longitudinal direction of the hardwoods or resin canals which may contribute to the continuous effective open area available for vapor movement in the longitudinal and radial directions of certain coniferous woods. For constant conditions with respect to other factors, the rate of vapor movement would vary directly as the second power of the radii of these openings.

Because of the anisotropic character of wood it seems desirable to consider moisture movement in the longitudinal direction more or less separate from movement in the radial and tangential directions. From figure 5, it is evident that there is a fairly definite linear relationship between the specific gravities of the various woods and the critical moisture contents for moisture movement in the longitudinal direction. By extending the straight line beyond the limits of the data, it is found to intersect the 100-percent line at a specific gravity of about 0.82. This indicates that the drying curve for a wood having a specific gravity of 0.82 or above, based on the green volume, would exhibit no constant rate period but rather would be comprised of only a falling rate period, at least when dried under the conditions employed in these experiments. When extended in the opposite direction, this straight line should pass through zero if the evaporation during the constant rate period is equivalent in character to evaporation from a free water surface; i. e., there should be no critical moisture content for evaporation from a free water surface. Extension of the curve on the basis of the equation derived by the method of least squares shows that it intersects the Y axis at +1.13 percent. Although this is in reasonable agreement with the theoretical result expected, based on the assumption that the rate of evaporation from the exposed surfaces of the blocks during the constant rate period is equal to the evaporation from a free water surface, there may be some significance to the slight deviation, as will be pointed out later.

The rate of evaporation during the constant rate period occurring in the drying of various solids quite generally has been assumed to be similar to the rate of evaporation from a free water surface. However, so far as the authors know, no attempt has been made to establish the magnitude of the difference in the rate of evaporation from a free water surface and the surface of a solid during the constant rate period. This has probably been due, in part, to the fact that data of sufficient reliability have not been available for the calculation ot the rate of evaporation from a free water surface under the conditions employed in the drying experiments. As shown in table 2, the rate of moisture loss during the constant rate periods was of the same order of magnitude for moisture movement in the longitudinal direction of the different woods as well as in the heartwood and sapwood of the same kind of wood. The rate ranged from 0.01110 g per hour per square centimeter for red pine sapwood to 0.01440 g per hour per square centimeter for basswood heartwood, with an average rate for all of the woods of 0.01285 g per hour per square centimeter. The data of Lurie and Michailoff (*14*) furnish a basis for a calculation of values to use in comparing these results with the rate of evaporation from a free water surface under comparable drying conditions. Because certain of the constants in the general equation given by these authors must of necessity incorporate and reflect to some degree the errors which existed in a number of relationships determined for a wide

range of drying conditions, it seemed desirable to employ a portion of their basic data and derive a new equation for drying conditions of a somewhat more limited scope. Since the air velocity of 1.1 meters per second employed in the experiments reported in this paper is the same as the air velocity used in the collection of the data for curve No. 1 of figure 9 in the paper by Lurie and Michailoff, it was easily possible to calculate a new equation for drying data of more limited scope than that utilized as the basis for their general equation. The rate of evaporation and the drying potential were determined for each plotted point used as the basis for curve No. 1 in figure 9 of their paper with the aid of a microscope and an eyepiece micrometer. The following linear equation then was derived by the method of least squares from these data:

$$Y=0.1376+0.03447\ X$$

in which, Y is the $\left(\frac{W}{ZF}\right)$ of Lurie and Michailoff or the rate of evaporation in kilograms per square meter per hour, and X is their $H-h$ or the drying potential in millimeters of mercury. Inserting into this equation the value for the drying potential calculated from Recknagel's formula as given by Lurie and Michailoff, a value of 0.01868 g per square centimeter per hour is obtained for the rate of evaporation from a free water surface under the conditions employed in the experiments. In the consideration of this result as the true rate of evaporation from a free water surface for the drying conditions employed, thought should be given to the fact that the straight line fitted to the data of Lurie and Michailoff by the method of least squares does not pass through zero as it should if the data are exactly correct and the relationship is linear throughout. However, the authors believe that the equation derived by the method of least squares from the data furnishes the more reliable basis for making an approximation of the rate of evaporation from a free water surface for the drying conditions employed in their experiments.

Obviously, the rate of evaporation obtained from the foregoing calculations is higher than 0.01285 g per hour per square centimeter, which was the average rate of evaporation observed during the constant rate period for moisture movement in the longitudinal direction of all of the woods included in the study. Furthermore, it is higher than the maximum rate of 0.01440 g per hour per square centimeter obtained during the constant rate period for moisture movement in the longitudinal direction of basswood heartwood. These results indicate that the rates of evaporation during the constant rate periods were lower than the rate of evaporation from a free water surface subjected to the same drying conditions. A similar indication, although slight, is furnished by the results for the relationship between critical moisture contents and specific gravities of the different woods; i. e., the straight line fitted to the data by the method of least squares does not pass through zero as it should if the evaporation during the constant rate period is equivalent in character to evaporation from a free water surface and if the relationship between the critical moisture content and specific gravity can be correctly assumed to be linear throughout its range.

It seems that a purely theoretical consideration of the problem also supports the contention that the rate of evaporation from wood

during the constant rate period should be less than the rate of evaporation from a free water surface subjected to the same drying conditions, although it need not be greatly less. A free water level would not be maintained at the surface of the wood throughout the constant rate period. The free water would evaporate from the open ends of the cut-off fibers, tracheids, or vessels, and meniscuses would be formed across the pit membrane pores and possibly across the openings in the perforation plates between vessel segments. These meniscuses might be maintained close to the surface during the constant rate period, but such an assumption hardly seems tenable when it is considered that 60 percent of the total removable water was lost from basswood and about 25 percent of it from hickory and bur oak during this period. In any event, the process of evaporation from the exposed water surfaces of the meniscuses would be appreciably retarded as a result of the decreased amounts of surface and the decreased vapor pressure at the water surfaces across small capillaries, particularly those across the pores in the pit membranes. The rate of evaporation from these surfaces could not be equal to the rate of evaporation from a free water surface having the same area as the exposed surface of the blocks. Under these conditions, water would undoubtedly move to the surface from the free water level either as bound liquid or as vapor or by a combination of these two mechanisms. Such movement could not take place, however, unless there was a moisture gradient set up in the wood and the surface layers were reduced to a moisture content less than the fiber-saturation point. Thus, although a constant rate of evaporation may be maintained during the evaporation of a large proportion of the total removable water, the rate of evaporation from the surface of the wood must be less than the rate of evaporation from a free water surface throughout all of the drying period with the possible exception of the rate of evaporation of a very small amount of water at the beginning of this period. The rate of evaporation decreases from the constant rate maintained during the loss of varying amounts of the total removable water from the different woods as soon as the rate of moisture movement to the surface no longer equals the rate of moisture loss during the constant rate period. The surface of the wood probably is maintained close to the fiber-saturation point during the constant rate period. The data shown in figures 2, 3, and 4 furnish evidence of a sudden increase in the resistance to moisture movement to the surface of the blocks at the termination of the constant rate period and the rate of moisture loss decreased very rapidly. It seems probable that this was due to the rapid development of a relatively dry surface layer of wood through which moisture must move prior to evaporation, the surface probably being reduced rapidly to a moisture content approaching equilibrium with the temperature and relative humidity conditions maintained in the drying cabinet. As drying continued, the dry surface layer became thicker so that the rate of moisture loss gradually decreased throughout the remainder of the drying period.

Although it must be recognized that some free water movement may have taken place during the constant rate period, the data obtained in this study support the view that the movement of moisture as free water was not of predominant importance for moisture

movement in the longitudinal direction during either the constant or the falling rate periods. The most important evidence obtained in support of this conclusion is furnished by the existence of the following: (1) The relationship between specific gravity and the critical moisture content. (2) The relationship between specific gravity and the rates of moisture loss when 50 and 25 percent of the total removable water remained in the wood. (3) The relatively rapid rates of moisture loss for movement in the radial and tangential directions in comparison with the rates of moisture loss for movement in the longitudinal direction when 50 and 25 percent of the total removable water remained in the wood.

The results of a number of studies made of the pressure permeability of the sapwood and heartwood of different kinds of wood have shown clearly that no universal correlation exists between pressure permeability and specific gravity for wood systems having such diverse characteristics. A consideration of the previously mentioned factors which influence free water movement shows that a correlation of this nature should be in evidence before one can reconcile the simultaneous existence of the first two relationships noted in the preceding paragraph, for movement of free water in the longitudinal direction to be of predominant or even appreciable importance.

Assuming there is a potential force of a comparable order of magnitude tending to cause intercellular free water movement in the different structural directions, the intercellular movement of water in this form should be much more rapid in the longitudinal direction than in the radial and tangential directions. The resistance to the intercellular movement of free water for a certain distance in the radial and tangential directions would be much greater than the resistance to movement for the same distance in the longitudinal direction because of the many more pit membranes in series. In addition, free water movement in the longitudinal direction of the hardwoods should tend to take place through longitudinal vessel systems. The resistance to intercellular movement through the pit membranes alone should be of the order of magnitude of 50 to 100 times greater for movement in the radial and tangential directions, than for movement in the longitudinal direction of woods such as those included in the investigation. On this basis alone, irrespective of the influence which the vessels might have on free water movement in the longitudinal directions of the hardwoods, the data given in table 2 show that the rates of moisture loss for movement in the radial and tangential directions as compared to movement in the longitudinal direction are much greater than those which would be expected if moisture movement in the form of free water was of appreciable importance. Apparently, the forces tending to cause free water movement in the longitudinal direction do not have the same opportunity to exert themselves as they do when the moisture moves in the radial and tangential directions, or the movement of moisture in the form of free water is not of appreciable importance for moisture movement in any of the different structural directions. More will be said about the possible importance of free water movement in the radial and tangential directions later in the discussion.

With the elimination of the possibility of free water movement as an important factor influencing the rate of moisture movement in the

longitudinal direction, there are, as previously mentioned, three other ways in which moisture can move from the interior of the wood to the surface, namely, as vapor, as bound liquid, or by a combination of vapor movement across the cell cavities and bound liquid movement through the cell walls. The presence of the rather definite relationships between the rates of moisture loss and specific gravity for the different groups of blocks comprised of heartwood and sapwood of different kinds of wood essentially eliminates the possibility of moisture movement from the interior of the wood to the surface predominantly in the form of vapor. It would be necessary to assume that the total effective cross-sectional area available for vapor diffusion bore the same relationship to specific gravity as the rates of moisture loss if one were to attempt an explanation of the observed relationships in terms of moisture movement from the interior of the wood to the surface predominantly in the form of vapor.

As was previously pointed out (*1*), Johnston and Maass (*12*) have found that the rate of pressure movement of water through Norway (red) pine sapwood was more than 200 times that for heartwood from the same tree. These results, of course, do not apply directly to movement of vapor through wood since the rate of vapor movement would vary as the second power of the radii of the effective openings while the pressure movement of water through wood varies as the fourth power of the radii of these openings. They do show, however, that there was a decrease in either the number or size of the openings present, or both. A decrease in either the number or size of the openings would result in a decreased rate of vapor movement from the interior of the wood to the surface. It is possible, although not probable, that the sapwood and heartwood of red pine could exhibit such decidedly different permeabilities from the standpoint of the pressure movement of water and at the same time be of essentially the same permeability from the standpoint of vapor diffusion. In order for this to be true, it is necessary to assume that there is an appreciable decrease in the size of the openings which would influence the pressure movement of water to a greater extent than it would vapor diffusion, and that there is a compensating increase, from the standpoint of vapor diffusion, in the number of openings during the change from sapwood to heartwood. Such assumptions do not seem tenable, particularly in view of the work of Griffin (*9*, *10*) and Scarth (*17*) which show that the more probable thing to expect is a decrease in the number of openings during the change from sapwood to heartwood as a result of an increase in the number of aspirated bordered pits. A decrease in the number of aspirated pits would influence the rate of pressure movement of water and vapor diffusion to the same relative extent. The decrease in number of openings, of course, also may be accompanied by a simultaneous decrease in effective size of the openings, this change influencing the two types of movement in the same direction but to the different degrees noted.

Extension of a general thought somewhat comparable to that presented in the comparison of red pine sapwood and heartwood to the hardwoods having different structural characteristics also yields evidence against the possibility of moisture movement in the longitudinal direction from the interior of the wood to the surface predominantly in the form of vapor. Bur oak heartwood is very impermeable to the movement of liquids and gases because the springwood vessels of this

wood are completely plugged with tyloses. The springwood vessels of red oak heartwood, on the other hand, do not possess tyloses and they can be readily penetrated by liquids and gases. Nevertheless, the rates of moisture loss for movement in the longitudinal direction, when both 50 and 25 percent of the total removable water remained in the wood, were less than those for bur oak, the difference in rate being in the direction expected on the basis of the relationships between rate of moisture loss and specific gravity shown in figure 6. When we compare the rates of moisture loss for moisture movement in the longitudinal direction of basswood and red pine heartwood, two woods having essentially the same specific gravity, we note differences in the rates of moisture loss in the direction expected on the basis of the relative opportunities for vapor movement from the interior of the wood to the surface. However, these differences are not of the magnitude which could be justly expected if moisture moved from the interior of the wood to the surface predominantly in the form of vapor. Stamm (*22*) has determined the continuous effective open areas for transverse sections of Sitka spruce, Alaska cedar, western red cedar, Douglas fir, and western yellow pine and found that they varied from 0.33 to 1.30 percent of the total membrane area tested, with an average value for the five woods of 0.66 percent. Although red pine heartwood was not included in this group, it seems reasonable to assume, when some consideration is given to results obtained by Erickson, Schmitz, and Gortner (*4*), that the continuous effective open area would be of somewhat the same general order of magnitude for this wood. French, as quoted by Forsaith (*7, footnote, p. 42*), has observed that the pores constitute approximately 55 percent of the cross section of basswood. The continuous effective open area will probably be less than this value because of some constriction at the point of coalescence of the vessel members. However, the vessel perforations of basswood are simple and the continuous effective open area would not be greatly less than the value observed by French since at least a small portion of the decrease in the continuous open area due to constrictions in the vessels would be compensated for by the contribution of the intercellular openings between the fibers to the total continuous effective open area. Consequently, although there is evidence of some moisture movement from the interior of basswood to the surface in the form of vapor, the results support the contention that the difference in the rates of moisture loss from basswood and red pine heartwood should have been much greater than that observed if moisture movement in the longitudinal direction is predominantly in the form of vapor.

The recent work of Stamm (*24*) furnishes data in confirmation of the findings of Johnston and Maass (*12*) and Buckman, Schmitz, and Gortner (*2*). Using a somewhat different method, Stamm likewise obtained evidence of a decrease in the effective size of the intercellular openings with increasing moisture content of the wood below the fiber-saturation point. In view of such information, one cannot account for the sudden decrease in the rate of moisture loss which was observed in the present study after the critical point had been reached in terms of the influence of moisture content on the size of the openings through which vapor movement can take place. The movement of moisture predominantly in the form of vapor would have

been facilitated rather than retarded by a rapid drying of the surface layers of the wood insofar as the movement through the intercellular openings is concerned, while in the case of movement through the vessels of the hardwoods, the results of another research of Stamm (*25*) show that there would have been little, if any, influence in either direction. Martley (*15*) has noted, however, that there is an appreciable decrease in the rate of moisture movement through wood with decreasing moisture content of the wood below the fiber-saturation point. It seems that this decrease must result from an appreciably increased resistance to moisture movement through the cell walls in the form of bound liquid which probably is caused by a decrease in the size of the capillaries between the structural units of the cell wall. If such is the case, a qualitative explanation is furnished for the sudden decrease in rate of moisture movement which figures 2, 3, and 4 show occurred with relatively little change in the moisture content of the entire block. Martley's results show that a sudden reduction in the moisture content of the surface layer of the block to approximate equilibrium with the relative humidity employed in the experiments would have created an appreciably increased resistance to moisture movement to the surface and would have caused a sudden change in the rate of moisture loss comparable in general nature to that observed.

Consideration of the data for moisture movement in the longitudinal direction with regard to possible evidence favoring the movement of water from the interior to the surface predominantly in the form of bound liquid shows that there is little, if any, evidence in support of such a view. Again, it must be recognized that the possibility of some bound water movement from the interior to the surface during the constant rate period cannot be completely eliminated on the basis of the available data. However, the existence of the three relationships shown in figures 5 and 6 seems to essentially eliminate the possibility of bound water movement in appreciable amounts throughout the entire drying process. Unless the data are interpreted in this manner, it is necessary to assume that the added amount of cell-wall substance present in the woods of higher specific gravity was not only totally ineffective in conducting bound water, but that it decidedly retarded the movement of moisture from the interior to the surface in the form of bound water. Despite the fact that a progressively larger proportion of the total removable water consisted of bound water in the case of the woods of higher specific gravity and the rate of moisture loss was undoubtedly reduced somewhat because of this factor, the foregoing assumptions are untenable and do not merit further consideration. This is particularly true in view of the relatively close agreement which Stamm (*23*) has observed between the fiber-saturation points of a number of different woods.

In accordance with the results of the preceding analysis, it seems that moisture must move in the longitudinal direction of wood predominantly by means of vapor movement across the cell cavities and bound liquid movement through the cell walls. Moisture movement from the interior of the wood to the surface in such a manner is in agreement with the relationships shown in figures 5 and 6, provided it can be correctly assumed that the rate of vapor movement across the cell cavities is appreciably faster than the rate of bound liquid

movement through the cell walls. The results of the calculations previously made by Buckman and Rees (*1*) show that the rates of vapor movement across the cell cavities and bound liquid movement through the cell walls were approximately in the ratio of 100 to 1 for the conditions of their experiment. In view of the results obtained by Martley (*15*), the relative rates of moisture movement through the cell wall would have been faster for the experiments reported in this paper than for those previously conducted by Buckman and Rees because of the higher moisture content of the cell-wall substance throughout the course of the later experiments. Study of all of the information available shows, however, that there still would be a pronounced difference in the relative rate of vapor movement across the cell cavities as compared to the rate of movement through the cell walls, the relative rates being of the order of magnitude of at least 30 to 1. The existence of differences between the rates of moisture movement in the two forms provide a semiquantitative explanation for the relationships shown in figures 5 and 6. With increasing specific gravity of the wood there will be a lengthening of the relative distance that moisture must move in the form of bound liquid, a shortening of the relative distance in which moisture must move in the form of vapor, and a decrease in the relative cross-sectional area of the cell cavities through which vapor diffusion can take place.

Moisture movement in the radial and tangential directions seems to be the result of the operation of a larger number of factors, at least in some instances, than in the case of moisture movement in the longitudinal direction. The data presented in figure 6 show deviations from a general relationship between specific gravity and the rates of moisture movement in the radial and tangential directions when 50 and 25 percent of the total removable water remained in the wood. By far the most outstanding deviation from the general relationship was observed for moisture movement in the tangential direction of basswood heartwood. The rate of moisture movement in the tangential direction of basswood heartwood was very rapid, the rates of movement in this structural direction being 90 and 75 percent respectively of the rate in the longitudinal direction when 50 and 25 percent of the total removable water remained in the wood. Although the rate of moisture movement in the tangential direction of basswood heartwood was the most outstanding deviation from the general relationship between rate of moisture movement in the radial and tangential directions and the specific gravities of the woods, it is apparent that there are definite variations in the results obtained for the sapwood and heartwood of the different kinds of wood which cannot be entirely explained by differences in specific gravity. The rates of moisture movement in the radial and tangential directions of red pine and silver maple sapwood were greater than those in the same structural direction of red pine and silver maple heartwood despite the fact that exact agreement with the rate of moisture movement-specific gravity relationship would require the reverse to be true or that no appreciable difference should exist as in the case of tangential movement in silver maple. These results combined with the available information on the permeability of sapwood and heartwood qualita-

tively indicate the existence of some degree of relationship between permeability and the rates of moisture movement in the radial and tangential directions of wood.

The foregoing indications of some degree of relationship between permeability of the wood and the rate of moisture movement in the radial and tangential directions may have been due to increased amounts of water moving from the interior of the wood to the surface either in the form of vapor or free water. Buckman and Rees (*1*) previously observed pronounced deviations from a relationship between specific gravity and rate of moisture movement in the radial and tangential directions of coniferous woods below the fiber-saturation point. Under the conditions of this experiment the possibility of free water movement was, of course, eliminated, and the deviation observed must have been due to moisture movement in the form of vapor to an increased extent in the case of a number of the woods. The data obtained in the present study do not permit one to evaluate the extent to which vapor movement from the interior of the wood to the surface contributed to the deviations from the rate of moisture movement—specific gravity relationships for moisture movement in the radial and tangential direction . The relative amount of moisture which may have moved to the surface in the form of vapor cannot be determined separately from the amount which may have moved to the surface in the form of free water. However, moisture movement in either form is dependent upon the number and size of the openings through which the movement can take place, the size of the openings influencing the two types of movement to the different degrees previously noted. Further experiments are being started to determine the relative importance of movement in the form of vapor and movement in the form of free liquid as the factors causing the deviations from the rate of moisture movement-specific gravity relationships for moisture movement in the radial and tangential directions.

SUMMARY

Blocks about 2.5 by 2.5 by 5.0 cm were prepared from six hardwoods and one softwood, including the heartwood and sapwood from the same log whenever possible. These blocks were slowly dried to equilibrium with the atmosphere of the room, in which condition they were kept until the beginning of the experiments. They then were soaked in water until completely saturated and subsequently coated with 16 coats of rubber latex in such a way as to confine the subsequent moisture movement to one structural direction of the wood. They then were exposed to a temperature of 30° C. and a relative humidity of 81 percent and were weighed at suitable intervals to determine the total moisture loss at the end of various periods of time. Using these data, the average moisture loss for the various sets of blocks was calculated, and curves were constructed showing the average moisture loss with time. The rates of moisture loss at various times during the drying process were determined from the slopes of tangents to moisture loss—time curves at regular intervals. The percentage of removable water remaining in the wood also was calculated at each point for which the tangent was constructed. From

these data, curves were drawn showing the rate of moisture loss versus percentage of removable water remaining in the wood.

All of the curves for the rate of moisture loss plotted against the percentage of the total removable water when movement was confined to the longitudinal direction were found to be divisible into two periods: (1) A constant rate period, and (2) a falling rate period. When the moisture movement was confined to the radial and tangential directions constant rate periods were observed for only silver maple sapwood, basswood heartwood, and in the case of radial movement, for red (Norway) pine sapwood.

The existence of the constant and falling rate periods was considered from the standpoint of the drying of wood. The rates of moisture loss during the constant rate period were of the same order of magnitude regardless of the kind of wood or the direction of flow, and information was presented supporting the view that the rate of moisture loss during the constant rate period was somewhat less than the rate of evaporation from a free water surface.

A linear relationship was found to exist between the critical moisture content (the percentage of the removable water remaining in the wood at the termination of the constant rate period) and the specific gravity of the different woods when the moisture movement was confined to the longitudinal direction.

The rates of moisture movement in the longitudinal direction varied from 1.2 to 25.5 times those in the radial and tangential directions when 50 percent of the total removable water remained in the wood. Although the differences between the rates of movement in the different structural directions were generally slightly less when 25 percent of the total removable water remained in the wood, the values were of a comparable order of magnitude.

The average rates of moisture movement in the longitudinal direction were 5.9 and 4.8 times those for moisture movement in the radial direction when 50 and 25 percent of the total removable water remained in the wood; and were 9.7 and 7.8 times those for moisture movement in the tangential direction when the same amounts of total removable water remained in the wood. The rates of moisture movement in the radial direction varied from 0.2 to 4.9 times those in the tangential direction, with respective average rates when 50 and 25 percent of the total removable water remained in the wood, of 1.9 and 2.0 times those in the tangential direction.

An exponential relationship was found to exist between the rate of moisture movement in the longitudinal direction and the specific gravity of the different woods when 50 percent of the total removable water remained in the wood. Evidence was presented that the same general type of relationship exists between the rate of moisture movement in the longitudinal direction and specific gravity when 25 percent of the total removable water remained in the wood. Likewise, the same general type of relationship seems to apply for moisture movement in the radial and tangential directions when 50 and 25 percent of the total removable water remained in the wood, although there were appreciable deviations from the general rate of moisture movement-specific gravity relationship for moisture movement in the radial and tangential directions.

The relationship between the critical moisture content and specific gravity and the relationships between rates of moisture movement and specific gravity for moisture movement in the longitudinal direction were discussed in the light of available related information and presented as information showing that moisture moves in the longitudinal direction of wood predominantly by bound liquid diffusion through the cell walls and vapor diffusion across the cell cavities.

The operation of the same general mechanism of moisture movement in the radial and tangential directions was supported by the available data. However, deviations from exact relationships between rate of moisture movement and specific gravity for moisture movement in the radial and tangential directions show that appreciable amounts of water moved from the interior of the wood to the surface either in the form of vapor or free water, or both. Evaluation of the relative importance of these two mechanisms of moisture movement was not possible from the available data, but the data showed that the opportunities for these two types of movement ultimately must be reconciled with the following: (1) The deviation from the general relationships between the rate of moisture movement and specific gravity for moisture movement in both the radial and tangential directions; (2) the lack of a general relationship between ray volume and the relative rate of moisture movement in the radial and tangential directions of different hardwoods; (3) the faster rate of moisture movement in the radial direction of red pine sapwood despite the almost complete absence of pits in the tangential walls of the tracheids; (4) a rate of moisture movement in the tangential direction of basswood which was five times the rate of movement in the radial direction, despite the relative uniform distribution of the vessels and of the pits on the radial and tangential walls of both the fibers and vessels of this wood; (5) the decreased values for the ratio between the rates of moisture movement in the radial and tangential directions in the case of silver maple and red pine sapwood as compared to the same ratios for silver maple and red pine heartwood.

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THE EFFECT OF NATURAL SELECTION IN A MIXTURE OF BARLEY VARIETIES [1]

By H. V. HARLAN, *principal agronomist*, and M. L. MARTINI, *assistant botanist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

For the past 13 years the writers have grown a mixture of barley varieties in an attempt to secure information on natural selection in barley (*Hordeum* spp.). Any analysis of changes in the population of plants in nature is beset with difficulties, for the factors that favor or discourage the best growth of species and strains are numerous and intangible. They cannot be isolated and tested one at a time under field conditions, and, since they vary from season to season, they are not readily measured except by the summary statement of census.

The problem explored in this experiment perhaps gained by its simplicity. Some of the factors that complicate the broader field of natural selection were avoided. The changes in population measured the reaction of a very limited number of pure lines of a single species, and no new forms were encountered during the test. The barley plants of the varieties used are much alike in their needs and competed for water and nutrients at the same time.

The experiment is not fully comparable to most population studies, because its original composition was wholly arbitrary. Since barley is self-fertilized, the competing types remained the same throughout the experiment. For this reason it affords an opportunity to observe the rapid elimination of types so unsuited that comparable sorts probably would not have been found in such an environment in nature. So far as the writers have been able to determine from the literature, the only similar experiment is that of Sapegin [2] with wheat, which was interrupted at the end of 4 years.

MATERIALS AND PROCEDURE

In 1923 seed of 11 varieties of barley was mixed together in such proportions that an equal number of plants of each might be expected. This mixture was sent to 10 experiment stations in the Northern and Western States to be grown in 1924. Through the kindness of men who had no direct interest in the project, the experiment was carried on for a number of years and is still being carried on at three stations.

The procedure at all points was essentially the same. The mixture was seeded at most places in field plots each year. These plots were harvested and threshed with the regular station equipment. Sufficient seed was saved to plant a plot the following year and to send a sample to Washington. These latter samples were space-planted in blocks at Aberdeen, Idaho, or Sacaton, Ariz., to determine

[1] Received for publication December 27, 1937; issued August 1938.

[2] SAPEGIN, A. A. [OBSERVATIONS ON THE CHANGES IN AN ARTIFICIAL MIXTURE OF TYPES.] Trudy Odesskoi Selsk. Khoz. Selek. Sta. Vyp. 6: 42-43. 1922. [In Russian.]

the number of plants of each of the 11 varieties in the population. At harvest time the space-planted plants were pulled and a census of 500 individuals was recorded. This number is used as the limit of population throughout the paper. The mixture consisted of Trebi, Coast, Hannchen, Manchuria, White Smyrna, Smooth Awn, Lion, Meloy, Svanhals, Gatami, and Deficiens. Nine of these varieties were easily identified, but all plants of Coast could not be distinguished readily from all plants of Trebi, so these two varieties are recorded together. This unfortunate choice of varieties obscured the trends in only three cases.

Counts were made for the maximum period of 12 years on the material from three stations. At some of the stations counts were not made for all years in which the mixture was grown. Such omissions are indicated by leaders in table 2.

EXPERIMENTAL RESULTS

The number of plants of each variety surviving in the final year at all stations is recorded in table 1. These are the end results of natural selection over a period of from 4 to 12 years. The response of a given variety to the environments afforded by the various localities is indicated by the number of plants of that variety surviving in the total population. A striking feature revealed in table 1 is the rapidity with which one or two varieties became dominant at certain places. This change was accompanied by an equally rapid elimination of other varieties. When considered in geographical sequence from east to west, omitting Arlington, Va., where winter-killing is a factor, the leading varieties are Manchuria, Hannchen, Trebi, White Smyrna, and Coast. The Trebi and Coast separation, although not made in the table, has been determined with sufficient accuracy to specify their fields of adaptation.

TABLE 1.—*Final census showing effect of natural selection in a mixture of barley varieties grown at 10 locations for 4 to 12 years, recorded as the number of plants of each of 11 varieties found in a population of 500 plants*

Variety	Number of plants of each variety in the year when last grown									
	Arlington, Va., 1928	Ithaca, N. Y., 1936	St. Paul, Minn., 1934	Fargo, N. Dak., 1930	North Platte, Nebr., 1932	Moccasin, Mont., 1936	Aberdeen, Idaho, 1936	Pullman, Wash., 1930	Moro, Oreg., 1934	Davis, Calif., 1928
Coast and Trebi	446	57	83	156	224	87	210	150	6	362
Gatami	13	9	15	20	7	58	10	1	0	1
Smooth Awn	6	52	14	23	12	25	0	5	1	0
Lion	11	3	27	14	13	37	2	3	0	8
Meloy	4	0	0	0	7	4	8	6	0	27
White Smyrna	4	0	4	17	194	241	157	276	489	65
Hannchen	4	34	305	152	13	19	90	30	4	34
Svanhals	11	2	50	80	26	8	18	23	0	2
Deficiens	0	0	0	1	3	0	2	5	0	1
Manchuria	1	343	2	37	1	21	3	1	0	0

A number of the varieties are of minor importance at all stations. Of these, Deficiens is least fitted to survive when all places are considered. Meloy is only slightly better. On the other hand, Hann-

chen, White Smyrna, and the Coast-Trebi combination are suited to a wide range of conditions. Since the Coast-Trebi totals are the sum of the number of plants of two varieties, the figures are too large to compare with those of single varieties, particularly when both Coast and Trebi are present in considerable numbers. However, the good showing of this combination at so many places is partly due to the fact that Coast is well adapted to the West, while Trebi is well adapted to the region from Idaho east. There is little Trebi left in the mixture in California and almost no Coast remaining at St. Paul and Ithaca. The environment of the stations chosen covers so wide a range that only Hannchen and the Coast-Trebi combination have survived at all of them. The elimination has been much more rapid at some places than at others. At Pullman, Wash., Arlington, Va., Moro, Oreg., and Davis, Calif., for instance, one or two varieties quickly dominated the population, whereas at Moccasin, Mont., North Platte, Nebr., and Aberdeen, Idaho, changes occurred more slowly and a greater number of varieties maintained a fair percentage of plants.

Some of the details are more apparent in table 2, where the yearly census is given for each station. In both tables there are two striking instances in which the dominant variety differs from that commonly grown by farmers in the vicinity. In New York State, Manchuria, a six-rowed sort, has become absolutely dominant, whereas most of the commercial acreage is in Alpha, a two-rowed variety similar to Hannchen. On the other hand, in Minnesota, where Manchuria and Manchuria hybrids constitute the greater part of the commercial acreage, Hannchen, a two-rowed variety, is predominant. Although two-rowed barleys are commonly grown around Ithaca, the preference for such varieties depends partly on considerations other than yield. Manchuria types produce about the same number of pounds per acre as the two-rowed, and since the seeds are smaller Manchuria is probably potentially able to produce a far greater number of plants. The behavior of Hannchen in Minnesota cannot now be fully explained. There are few, if any, fields of Hannchen grown in the northern Mississippi Valley. The variety does occur, however, as a mixture in fields, and the percentage has increased in the past 30 years. Hannchen lodges more than Manchuria and is not desired by the market, but solely from the standpoint of plant productivity it would appear that it could be successfully grown.

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Aberdeen, Idaho:												
Coast and Trebi	97	104	130	136	187	159	216	214	218	176	198	210
Gatami	49	32	34	34	30	20	11	15	12	17	11	10
Smooth Awn	43	40	31	18	10	6	2	7	3	1	0	0
Lion	40	45	24	40	17	21	6	10	5	3	3	2
Meloy	37	34	26	22	8	9	10	12	7	2	2	8
White Smyrna	65	51	92	89	86	119	145	136	154	160	177	157
Hannchen	59	81	80	80	111	109	82	76	75	106	75	90
Svanhals	33	48	31	39	25	33	23	15	22	28	20	18
Deficiens	40	21	18	14	13	7	2	9	2	2	8	2
Manchuria	37	44	34	28	13	17	3	6	2	5	6	3

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*—Continued

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Ithaca, N. Y.:												
Coast and Trebi [1]	107	64	93	79	70	75	74	82	77	60	61	57
Gatami	45	34	62	53	69	46	33	40	28	31	22	9
Smooth Awn	45	43	50	43	64	47	72	51	46	51	43	52
Lion	34	27	31	34	32	44	29	30	15	19	6	3
Meloy	36	9	5	6	4	0	0	1	0	0	0	0
White Smyrna	25	16	14	7	5	1	1	0	4	1	0	0
Hannchen	75	77	75	79	55	17	11	25	38	43	62	34
Svanhals	35	52	40	47	14	8	3	6	4	5	4	2
Deficiens	43	40	12	3	1	0	0	0	0	0	0	0
Manchuria	55	138	118	149	186	262	277	265	285	290	302	343
Moccasin, Mont.:												
Coast and Trebi	105	106	93	94	83	102	110	106	81	75	96	87
Gatami	55	64	72	56	64	73	62	79	87	88	78	58
Smooth Awn	55	52	42	48	54	54	39	43	37	37	39	25
Lion	39	48	44	26	31	44	48	34	33	49	47	37
Meloy	55	35	24	29	14	12	11	14	7	5	5	4
White Smyrna	34	46	54	47	59	89	108	120	187	175	180	241
Hannchen	34	39	57	45	75	55	44	55	24	29	22	19
Svanhals	35	34	45	41	40	31	32	19	9	9	6	8
Deficiens	37	14	7	14	10	2	5	1	0	1	0	0
Manchuria	51	62	62	100	71	38	41	29	33	32	27	21
North Platte, Nebr.:												
Coast and Trebi	130	107	.....	164	139	176	229	224	.....	.....	.....	.....
Gatami	65	48	.....	49	68	46	17	7	.....	.....	.....	.....
Smooth Awn	45	38	.....	26	24	20	5	12	.....	.....	.....	.....
Lion	29	48	.....	29	18	24	27	13	.....	.....	.....	.....
Meloy	44	59	.....	34	26	11	10	7	.....	.....	.....	.....
White Smyrna	51	46	.....	73	118	149	172	194	.....	.....	.....	.....
Hannchen	44	48	.....	40	33	22	8	13	.....	.....	.....	.....
Svanhals	26	48	.....	25	38	35	21	26	.....	.....	.....	.....
Deficiens	26	10	.....	21	10	4	6	3	.....	.....	.....	.....
Manchuria	40	48	.....	39	26	13	5	1	.....	.....	.....	.....
Fargo, N. Dak.:												
Coast and Trebi	86	.....	113	113	152	156	.....	.....	.....	.....	.....	.....
Gatami	56	.....	60	40	35	20	.....	.....	.....	.....	.....	.....
Smooth Awn	50	.....	47	31	42	23	.....	.....	.....	.....	.....	.....
Lion	47	.....	38	28	9	14	.....	.....	.....	.....	.....	.....
Meloy	30	.....	9	5	2	0	.....	.....	.....	.....	.....	.....
White Smyrna	39	.....	42	46	19	17	.....	.....	.....	.....	.....	.....
Hannchen	58	.....	62	102	117	152	.....	.....	.....	.....	.....	.....
Svanhals	66	.....	75	86	77	80	.....	.....	.....	.....	.....	.....
Deficiens	13	.....	2	2	2	1	.....	.....	.....	.....	.....	.....
Manchuria	53	.....	52	47	45	37	.....	.....	.....	.....	.....	.....
Moro, Oreg.:												
Coast and Trebi	147	.....	126	.....	148	125	.....	67	24	6	.....	.....
Gatami	56	.....	81	.....	15	3	.....	0	0	0	.....	.....
Smooth Awn	59	.....	38	.....	15	10	.....	0	0	1	.....	.....
Lion	109	.....	25	.....	21	3	.....	0	0	0	.....	.....
Meloy	60	.....	35	.....	11	3	.....	0	0	0	.....	.....
White Smyrna	21	.....	57	.....	192	276	.....	394	458	489	.....	.....
Hannchen	11	.....	69	.....	60	48	.....	39	8	4	.....	.....
Svanhals	15	.....	28	.....	33	26	.....	0	0	0	.....	.....
Deficiens	11	.....	18	.....	1	0	.....	0	0	0	.....	.....
Manchuria	11	.....	23	.....	4	6	.....	0	10	0	.....	.....
St. Paul, Minn.:												
Coast and Trebi [1]	110	99	121	94	122	121	.....	99	117	83	.....	.....
Gatami	40	50	59	34	31	16	.....	5	23	15	.....	.....
Smooth Awn	61	106	65	51	55	37	.....	16	33	14	.....	.....
Lion	48	61	56	52	51	34	.....	26	19	27	.....	.....
Meloy	64	22	9	9	7	5	.....	2	2	0	.....	.....
White Smyrna	28	16	14	11	14	5	.....	3	11	4	.....	.....
Hannchen	56	59	105	149	147	215	.....	251	248	305	.....	.....
Svanhals	32	34	49	69	63	57	.....	93	38	50	.....	.....
Deficiens	14	10	1	4	1	0	.....	0	0	0	.....	.....
Manchuria	47	43	21	27	9	10	.....	5	9	2	.....	.....
Davis, Calif.:												
Coast and Trebi [2]	134	251	338	362	.....	.....	.....	.....	.....	.....	.....	.....
Gatami	29	8	4	1	.....	.....	.....	.....	.....	.....	.....	.....
Smooth Awn	19	13	7	0	.....	.....	.....	.....	.....	.....	.....	.....
Lion	34	29	14	8	.....	.....	.....	.....	.....	.....	.....	.....
Meloy	81	43	40	27	.....	.....	.....	.....	.....	.....	.....	.....
White Smyrna	46	65	33	65	.....	.....	.....	.....	.....	.....	.....	.....

[1] All or nearly all Trebi at Ithaca, St. Paul, and Arlington.
[2] All or nearly all Coast at Davis.

TABLE 2.—*Annual census of 11 barley varieties grown as a mixture at 10 stations for 4 to 12 years as determined by the identification of varieties in samples of 500 plants*—Continued

Station and variety	Number of plants and year of identification											
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Davis, Calif.—Contd												
Hannchen	69	46	40	34								
Svanhals	44	24	15	2								
Deficiens	13	17	8	1								
Manchuria	31	4	1	0								
Arlington, Va.:												
Coast and Trebi [1]		253	387	446								
Gatami		62	23	13								
Smooth Awn		27	15	6								
Lion		53	19	11								
Meloy		18	6	4								
White Smyrna		28	10	4								
Hannchen		10	7	4								
Svanhals		40	20	11								
Deficiens		0	0	0								
Manchuria		9	13	1								
Pullman, Wash.:												
Coast and Trebi					159	150						
Gatami					2	1						
Smooth Awn					5	5						
Lion					10	3						
Meloy					10	6						
White Smyrna					230	276						
Hannchen					42	30						
Svanhals					28	23						
Deficiens					10	5						
Manchuria					4	1						

[1] All or nearly all Trebi at Ithaca, St. Paul, and Arlington.

DISCUSSION

Survival in competition seems to be dependent on two factors which are summaries of various unknowns. The number of plants of a given variety that will be present in any year will depend on the number (not the weight) of seeds sown and on the percentage of survival of the seedlings in competition. For theoretical purposes this may be reduced to a single mathematical factor, namely, the number of seeds produced that will produce plants the following year. This ignores some factors that are known to be present and that would affect the trends, but it does give a working basis for a calculating-machine experiment in the office. Postulating 10 varieties, the poorest of which produces 45 seeds per plant, the best, 90 seeds, and the remaining 8 separated by intervals of 5 seeds each, we would obtain the curves shown in figure 1. In this figure all populations are limited to 500 individuals.

These curves, being unhampered by effects of temperature, rainfall, and pests, are very regular. The poorest variety is eliminated promptly. The best one increases for several years at a rate that, when plotted as a curve, closely approximates a straight line. The better sorts increase as long as part of their competition is against poorer varieties. Even among those moderately poor the decrease is retarded for a year or two, while the least productive varieties are still represented by a fair proportion of plants. The second best variety does not start to decrease until one-half of the total population is made up of plants of the best variety. Eventually only one

variety would be left, but the last plants of all varieties are slow to disappear. When the population has reached the point where the best variety has 498.1 plants and the second best 1.9 plants, 10 years more are required to reduce this fraction below 1.0. Assuming that less than two plants is one plant, and less than one plant no plant, it would require 10 years to eliminate the last plant.

These theoretical curves include an obvious fallacy. It is reasonable to suppose that the number of seeds produced by the best variety would be greater in the beginning, when the poorer varieties still constituted a considerable part of the population, than later when the competition is greater. An experiment is now being devised to measure this influence, but until definite information is available it does not seem worth while to introduce the refinement of a hypothetical factor. As a matter of fact this additional factor, while undoubtedly present, is probably of minor importance. It would change the pitch of the curves, but not the type, and it is the type that is here important rather than absolute fit.

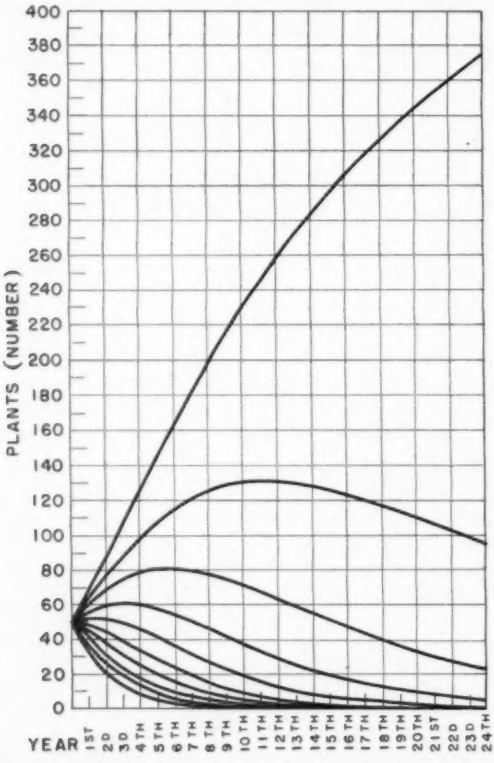

FIGURE 1.—Theoretical curves of natural selection based on an equal mixture of 10 varieties differing by 5 kernels each in their productivity per plant, the poorest plant producing 45 seeds.

The theoretical curves vary in their pitch according to the percentage difference in the intervals. The actual ones are based on many variables. In the first place, no set of 10 varieties can be chosen that are separated by equal intervals in their productivity as measured by number of seeds per plant. Also, since the climate of the United States is a continental one, the seasons favor first one variety and then another. This influence of climate may or may not be sufficient to change the order of the leading types, but in all cases it causes variations in the trend. The most exaggerated instance is Hannchen at Ithaca. At the beginning of the experiment conditions were favorable to Hannchen. Then came a series of years in which the percentage of Hannchen decreased rapidly. When the population had been

reduced from 79 to 11 plants a series of more favorable years ensued, and this variety slowly increased, reaching 62 before the onset of unfavorable conditions brought about another decline. The fluctuations of Hannchen did not, however, seriously affect the trend of the dominant variety.

It is remarkable that the curve of the Hannchen variety grown at Ithaca is the only one definitely bimodal. There are other instances of large annual fluctuations. Naturally, in the field, with widely varying seasons, uniform trends cannot be expected. At Moro, Oreg., for example, the first few years were favorable to Coast and Trebi. Then came a series of years during which Smyrna maintained an increase that charts as a nearly straight line. At Moccasin, Mont., the year 1926 was favorable for the development of Manchuria barley, and in consequence the 1927 population counted in 1928 showed a remarkable increase. The percentage of this variety declined after that time. Even in these extreme cases the fluctuations have not obscured the trends. The question is, Do the trends approach the theoretical ones? Are the actual curves of the same type as those shown in figure 1?

Three types of curves appear in figure 1, the two simple ones of the best and poorest varieties, and the humped one of the intermediate varieties. The curves of the three or four poorer varieties are very similar, differing mostly in the time required for complete elimination. It must be kept in mind that the curves are based on the number of plants rather than on percentage intervals, but nature deals with plant units. In the actual data obtained many instances of curves of rapid elimination were found. Such trends are shown by Deficiens at all 10 stations, by Meloy at Ithaca, Fargo, and St. Paul, by Smooth Awn at Aberdeen and Davis, by White Smyrna at Ithaca, and by Manchuria and Gatami at Davis. There are thus 18 or more curves of this general pattern. One of these is illustrated in figure 2, where a typical curve can be drawn through 10 of the 12 points of Meloy at Moccasin, Mont. The departures in this case seem to be due to errors of sampling rather than to seasonal fluctuations. This conclusion is based on the fact that the number of plants of this variety the following year is in line with the point where the theoretical line passed rather than on the point fixed by the census; that is, the number of plants in the population of 1929 is just what it should have been if in 1928 there had been the theoretical 18 instead of the recorded 29 plants.

Theoretically, the best variety at any place will eventually dominate the population, and the increase in numbers of the best variety is shown as a nearly straight line when plotted. The interval between the curves of the two best varieties is much greater than that between the curves of the two poorest varieties. In the counts a single variety with the characteristic dominant trend is found at most places. Typical illustrations are Hannchen at St. Paul, Manchuria at Ithaca, White Smyrna at Moccasin, Moro, and Pullman, and Coast at Davis. At Aberdeen, Fargo, and North Platte the situation is confused by the inability to separate Coast and Trebi, but with these exceptions there is no question which variety is dominant at any place. The actual data for Hannchen at St. Paul are plotted along with the theoretical curve in figure 3. The agreement is striking.

The intermediate curves shown in figure 1 may be expected to fall into two classes when applied to the actual data. Inasmuch as the seasonal fluctuations cause a degree of uncertainty in the actual trend, the five poorest varieties would not readily be separable. This is indicated in the large number already enumerated, which follow curves of rapid elimination. There then remain two curves of longer period of elimination, but more nearly approaching a straight line, and two that are definitely humped. These humped curves represent the better varieties, and the actual counts should first increase and then decrease.

The curves in figure 1 are the result of computations for 25 years. At most places a much greater interval is indicated in the actual

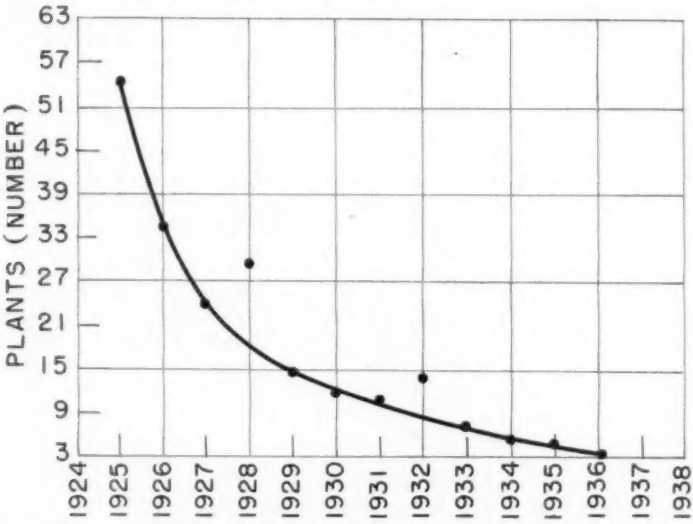

FIGURE 2.—Data on the Meloy variety at Moccasin, Mont., showing typical curve of poor variety.

data than was used in figure 1. The clearest trends in the short period of 12 years naturally came from the stations where the changes are rapid. At one or two places there are varieties in which the trend is only beginning to become definite. Definite increases in the first or later years followed by subsequent recessions are apparent in Lion at Aberdeen, Ithaca, and Moro, in Gatami at Ithaca and North Platte, in Hannchen at Moccasin and Moro, and in Smooth Awn, Manchuria, and Svanhals at Moccasin.

Data from other experiments in the field show that among varieties of nearly the same productivity the order of yield varies from year to year. The difficulty lies in determining what is fluctuation and what is trend, and how fair it may be to emphasize those curves that parallel most closely the theoretical. If carried a sufficient number of years, Smyrna and Hannchen at Aberdeen may prove to be perfect examples of nearly dominant varieties that are taking the long road to extinction. Svanhals at Aberdeen shows the trend of an average variety, with little early increase and a long period of elimination.

While not reproduced here, a good agreement of actual trend with theoretical is found if the computations are based on an interval less than that shown in figure 1. Svanhals at Moccasin follows a similar curve with a more rapid elimination. The data obtained at Moccasin are plotted in figure 4. The actual trend quite closely parallels the theoretical. In many cases good intermediate curves of greater initial increase are obtained if the data are smoothed with a moving average. Two of these, Hannchen and Manchuria at Moccasin, are shown in figures 5 and 6. In both cases the theoretical was computed with a greater interval than that used as a base in figure 1. In figure 6 it is obvious that a still greater interval would have been better. No attempt has been made in any case to find the curve that would best fit the data. This experiment is preliminary, and if the data so far obtained indicate the type of curve that may be expected, further refinements can well await the accumulation of more extensive data.

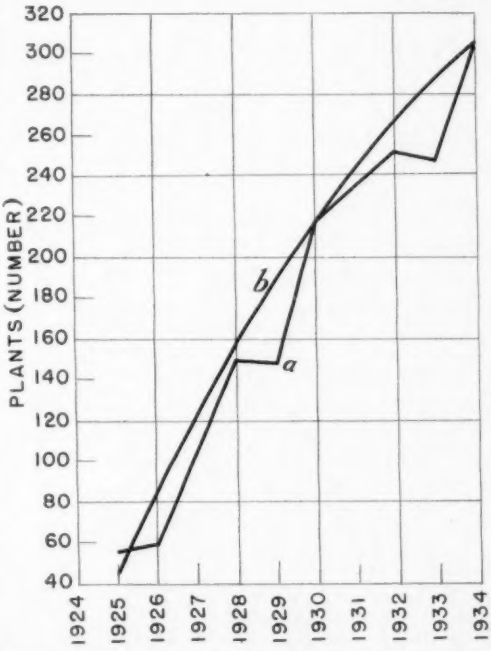

FIGURE 3.—Curve (*a*) of actual data of Hannchen at St. Paul, Minn., compared with the theoretical curve (*b*) of a dominant variety.

Naturally, many defects appear in the course of an experiment. The most important in the present work is the absence of any method of interpreting fluctuations. A new experiment has been planned that may be one step farther along, but a full understanding of all the factors that influence the results will never be achieved. A dry or a wet season may favor one variety or another. Rain on a given day may favor one variety much more than another, even though their total moisture requirements are the same. High temperatures for a few critical days may mean more than the average maximum or the absolute maximum. Some of the variables can be eliminated, others may be interpreted eventually, but many can only be accepted.

SUMMARY

A mixture of 11 varieties of barley was grown at 10 stations for a period of 4 to 12 years. The mixture was seeded, harvested, and threshed with the ordinary station equipment. Seed as it came from the thresher was saved for seeding the following year. Population counts were made annually.

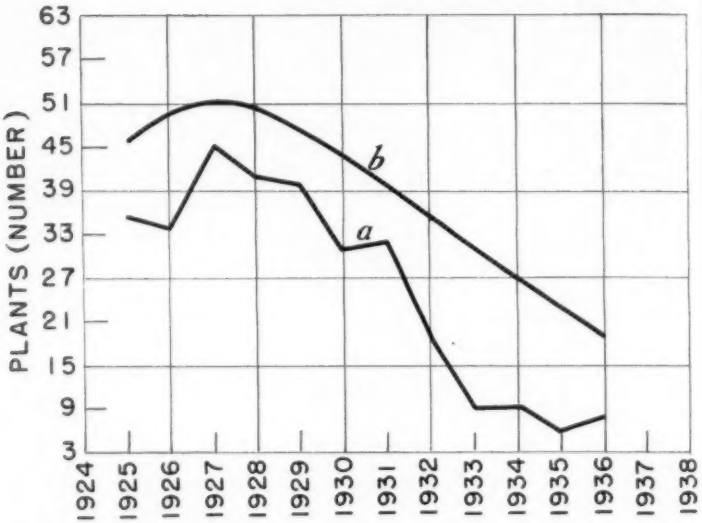

FIGURE 4.—Curve (*a*) of actual data of Svanhals at Moccasin, Mont., compared with the theoretical curve (*b*) of a variety slightly better than average.

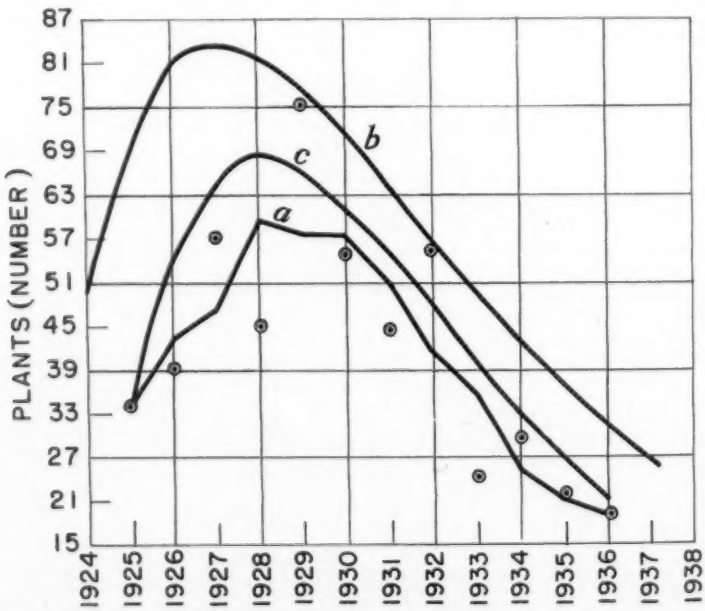

FIGURE 5.—Actual data of Hannchen at Moccasin, Mont., shown as unconnected points: *a*, The same data smoothed with a moving average; *b*, a theoretical curve as calculated for a better-than-average variety; *c*, the same curve superimposed by applying its first point to coincide with the census of 1925.

At all places there was a rapid elimination of the less adapted sorts. At most places the variety that would eventually dominate the population was quickly evident. The leading variety varied with the location of the station. A variety dominant at one station was

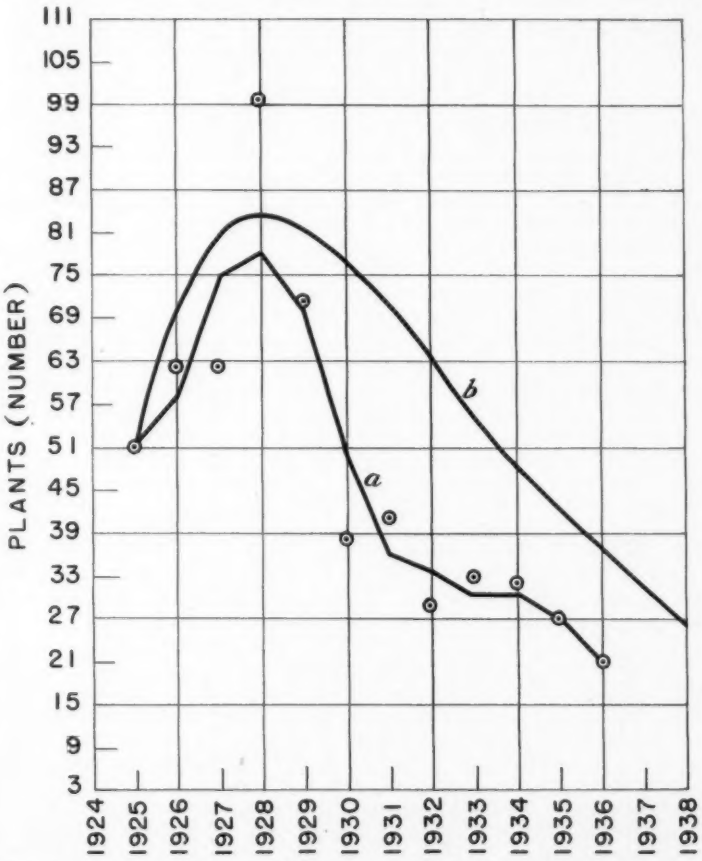

FIGURE 6.—Actual data of Manchuria at Moccasin, Mont., and the same data smoothed with a moving average (*a*) compared with a theoretical curve (*b*) of a good variety.

eliminated at another. Few varieties survived at all stations. Some varieties increased for a time and then decreased.

The population trends in general agree with a series of theoretical curves here presented. At all places the poorest varieties show the same type of descending curve. The best variety exhibits a typical ascending curve, which for a time approaches a straight line. Trends of many of the intermediate varieties are of the same type as the compound curve suggested in the theoretical scheme, first increasing, then decreasing.

EFFECT OF NEUTRAL SALTS OF SODIUM AND CALCIUM ON CARBON AND NITROGEN OF SOILS[1]

By V. P. SOKOLOFF

Formerly *junior agronomist, Division of Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Saline waters are frequently employed for irrigation purposes in the arid and semiarid regions of the Southwest. The amounts and the kinds of salts carried by irrigation waters determine to some extent the concentration and composition of the soil solution of irrigated lands. Considerable attention has been devoted, accordingly, to the study of the effects of salts and of individual ions on the physicochemical characteristics of soils. Comparatively little systematic interest has been manifested, however, in ascertaining the influence of neutral salts on the microbiological transformations of the organic matter of soils such, for example, as lead to the production of carbon dioxide and nitrates.

The conclusions of Lipman (*13*)[2], Kelley (*12*), Greaves (*11*), and Gedroiz (*8*) show a degree of accord as to the possibility of stimulating effects of small amounts of neutral salts of sodium upon nitrification in soils, at least under certain conditions. Stimulation of the nitrification processes in the presence of small amounts of neutral salts of calcium also has been reported (*13*). Higher concentrations of the neutral salts of calcium have been regarded as being more unfavorable to nitrification than corresponding concentrations of the neutral salts of sodium (*16*). The favorable effect of the oxides or carbonates of calcium on nitrification has often led to the opinion that the calcium ion, as such, is a stimulant to nitrification (*15*, *16*). Absorbed calcium, in the absence of other absorbed cations, has been shown to retard nitrification processes in the soil (*4*).

Several years ago, in an attempt to correlate the amounts of salts added to the soil, in the course of successive wetting and drying treatments, with the composition and concentration of the corresponding displaced soil solutions, the author's interest was aroused by certain fairly consistent trends in the nitrate content of displaced soil solutions. The addition of moderate amounts of sodium chloride to the soil (up to 6 milliequivalents per kilogram) was frequently followed by an increase in the nitrate concentration of the soil solution, while the addition of equivalent amounts of calcium chloride seemed to have the opposite effect. The correctness of the observations in regard to the stimulating effect of sodium chloride upon the nitrification processes of the soil was verified by simple incubation tests. At the same time the technique of the present investigation was developed. Among other things, the length of contact between soil and water during the extraction came to be regarded as an important variable,

[1] Received for publication April 25, 1938; issued August 1938. Based on a thesis submitted to the University of California in partial fulfillment of the requirements for the degree of doctor of philosophy, March 1937.

[2] Italic numbers in parentheses refer to Literature Cited, p. 215.

capable of influencing the nitrate and bicarbonate content, as well as the hydrogen-ion concentration of the extracts, especially in the presence of organic matter and of sodium salts. Periodical removal of the accumulated products of microbiological activities was likewise recognized as an important feature of the nitrification studies.

The investigation herein reported was undertaken to determine, by means of the foregoing technique, the influence of neutral salts of sodium and calcium on carbon and nitrogen transformations in two natural soils and in a third soil prepared by leaching with sodium chloride a portion of one of the original soils.

REVIEW OF LITERATURE

Lipman (*13*) observed that the nitrification processes in the soil are favored by the addition of either sodium chloride or sodium sulphate. A significant increase in the amounts of nitrate, according to Lipman, took place in the presence of 0.05 to 0.10 percent of either of these sodium salts in a soil containing 2 percent of dried blood. Higher concentrations of the salts inhibited the nitrification processes. Kelley (*12*) found that the addition of sodium sulphate to the soil stimulated nitrification under certain conditions. For example, 0.1 percent of sodium sulphate favored the nitrification of 1 percent of dried blood in a manured plot, while the same amount of the salt had no effect on the nitrification of 0.1 percent of dried blood in a control plot. The addition of ammonium sulphate to the manured plot containing 0.1 percent of sodium sulphate had no effect on the nitrate production. In the control plot, on the other hand, the nitrification of ammonium sulphate was retarded by sodium sulphate. The results of Greaves et al. (*11*) confirmed the observations of Lipman (*13*) in regard to the stimulating effect of sodium chloride on the nitrification of dried blood. Greaves and his associates, however, observed no stimulation in the presence of sodium sulphate, while calcium sulphate proved to be a stimulant within the entire range of the concentrations studied. The findings of these investigators suggest that the stimulation of nitrification, wherever it occurs, is due to the effect of individual salts on individual soils rather than to the specific influence of individual ions. The findings of Lipman and of Greaves et al. are not in agreement with the earlier results of Dehérain (*6*) nor with the conclusions of Nehring and Möbius (*15*) in a more recent investigation. It may be observed, however, that the generalizations of Nehring and Möbius, insofar as they deal with the depressing effect of sodium and potassium ions, are based on the study of the effect of sodium and potassium carbonates. The toxicity of relatively small quantities of sodium carbonate to the nitrifiers of the soil was recognized in all investigations previously discussed (*11, 12, 13*). The possibility of more rapid mineralization of the organic nitrogen of the soil in consequence of the addition of sodium salts to Chernozem soils initially free from sodium was indicated by Gedroiz (*9*). According to Gedroiz, the "optimum mobility" of the soil nitrogen may be attained when the soil solution becomes 0.01 to 0.1 normal with respect to sodium chloride, provided the air and water regimes of the soil are favorable for plant growth.

Chizhévsky (*4*), in his studies of the effect of absorbed cations upon the decomposition of organic matter in soils, found that the production of carbon dioxide was markedly stimulated by absorbed sodium and,

conversely, was depressed by absorbed calcium. The sodium-saturated soils exceeded all others in the density of their bacterial population. While both nitrification and ammonification were retarded in the calcium-saturated soils as compared with the untreated soil, no nitrogen data were reported by Chizhévsky in the case of the sodium-saturated soils. He believes, on the basis of his results with the iron-, hydrogen-, and calcium-saturated soils, that there is no parallelism between the accumulation of nitrates and the production of carbon dioxide. It may be argued, however, from Chizhévsky's own findings, that the hydrogen- and the calcium-saturated soils are not comparable among themselves, inasmuch as they differ radically in their microflora; the fungi were the only prominent group of micro-organisms that developed in the hydrogen-saturated soils, while the calcium- and the sodium-saturated soils, on the other hand, were found to be entirely comparable in this respect.

While investigators agree as to the probability of the stimulating effects of neutral sodium salts on nitrification, at least under certain conditions, the effect of the corresponding calcium salts seems to be a matter of some controversy. Waksman (*16*) states that the salts of calcium and magnesium are much more injurious to nitrification than the salts of sodium and potassium. Greaves (*11*) reports stimulation in the presence of very small quantities of calcium chloride. Nehring and Möbius (*15*) conclude that nitrification is stimulated by the calcium ion as such. It may be pertinent here to cite the observation of Kelley (*12*) that "almost any kind of conclusions may be drawn regarding the relative rates of nitrification * * *, provided that the incubation periods be carefully chosen." In order to overcome this fundamental weakness of all nitrification studies, Kelley recommends variations in the length of the incubation periods.

The effect of the neutral sodium salts on the production of carbon dioxide in the soils has received practically no attention. The greater solubility of the soil organic matter in the presence of sodium is, on the other hand, a recognized fact (*16*, *17*). The fundamentals of present knowledge in regard to nitrification and carbon dioxide production in soils are incorporated in textbooks (*7*, *16*, *17*), and need not be reviewed here. It may be remarked, however, that the older investigators paid no attention to the kind and quantity of salts inevitably present in the organic materials they have been adding to the soil and that they gave no consideration to the possibility that these salts, even in small quantities, might play a part in the transformations of the soil organic matter.

METHODS AND MATERIAL

EXPERIMENTAL PROCEDURE

Throughout the experiments 1-kg portions of soils were used. The soils were incubated at one and one-half times the moisture-equivalent moisture in parallel series, with and without artificial aeration, for several successive 15-day periods. Each period was preceded and followed by a 1-to-1 extraction. Conclusions as to the effects of the electrolytes on the transformations of organic matter in the incubated soils were based on the analyses of the 1-to-1 extracts and on the determinations of carbon dioxide and ammonia volatilized under aerated conditions.

One-kilogram aliquots of air-dried soils were extracted each with 1 liter of a N/20 electrolyte.[3] (One-tenth normal electrolytes were used with the third soil.) Distilled-water controls were included with every set of treatments, except with soil F-Na, as explained later. The extracts were separated from the soil by filtration through a double layer of Whatman No. 2 filter paper on Büchner filters. The extracts were returned to the filters repeatedly to insure uniformity of composition. The suction was continued until no more filtrate was obtained. The clear final filtrates, designated as the "zero time," were reserved for analysis. The extracted soils, generally retaining moisture corresponding to one and one-half times the moisture equivalent of the given soil, were passed through a 2-mm screen and packed loosely into 2-liter aeration flasks in such a manner as to occupy about 1½ liters of space per kilogram of the soil. The flasks containing the weighed moist soils were then aerated at room temperature for 10 hours a day at the rate of about 180 cc per minute. Day temperatures varied between 22° and 27° C.; during the night the temperature sometimes dropped below 20°.

After 15 days of such intermittent aeration the moist soils were weighed and extracted for the second time, following the technique previously indicated. Each experiment consisted of three successive 15-day aeration periods, totaling 45 days, and each of these periods was preceded and followed by a 1-to-1 extraction with a N/20 electrolyte. Parallel series of experiments were conducted with the soils incubated in stoppered jars. These unaerated soils were packed so as to occupy no more space than 0.9 liter per kilogram of soil. At the conclusion of the experiments the soils were dried in the air for 6 weeks before analysis.

The moist weight of the soils after the extraction afforded a basis for estimating the residual amounts of the dissolved substances that were carried over from period to period, on the assumption that the clear filtrate and the moisture retained by the soil were identical in composition as regards the dissolved substances. Changes in the moist weight of the soils during the aeration were taken into account in the extraction calculations. The extractions required from 1 to 6 hours each, and the subsequent screening and packing of the extracted soils required an additional 40 minutes. Two of the soils used in these investigations remained permeable to N/20 sodium chloride through the five successive extractions; in preliminary tests, difficulties had been encountered when more dilute solutions were tried. The permeability of soil F-Na was altered by presaturation with sodium; it was therefore necessary to use N/10 concentrations of the electrolytes in order to obtain a satisfactory rate of filtration, and the distilled-water series had to be omitted because of the imperviousness of the soil to water.

AERATION APPARATUS

The aeration apparatus consisted of the following units arranged in the following order:

(1) Air scrubbers: Two 2-foot towers with glass beads, inserted into 1-liter reservoir bottles containing 40 percent of sulphuric acid and 40 percent of sodium hydroxide, respectively.

[3] Calcium sulphate was used in the form of a suspension containing 50 milliequivalents of the salt per liter.

(2) One 3-foot water tower filled with beads, connected with the air scrubbers and, by means of a glass manifold tube, with the aeration flasks.

(3) Four aeration chambers: The moist air, free from carbon dioxide and ammonia, entered the flasks at the bottom through a wide glass tube and passed upward through the soil mass. A current of air was thus formed into which the gases released by the soil could diffuse. This means of aeration was preferred to other methods when the aeration periods were long.

(4) Carbon dioxide-absorbing towers: Each aeration flask was connected to a long-stemmed, fine-tipped 100-cc pipette, calibrated as to the delivery time, the tip of which touched the bottom of a reservoir flask containing 75 cc of N/2 sodium hydroxide. The incoming air lifted the alkali of the reservoir into the pipette. The small bubbles of air moving upward through the pipette and coming in contact with the film of alkali streaming down the narrow walls of the stem of the pipette were freed from carbon dioxide by the time they entered the next unit of the aeration chain.

(5) Ammonia-absorbing towers containing N/2 hydrochloric acid were constructed in the same way as the carbon dioxide absorbers. The outlets of these units were connected with the needle-valve manifold, and the outlet of the latter with the vacuum pump.

All connections of the aeration apparatus were fitted airtight and sealed with wax. Only the necessary minimum of rubber tubing was used, and all rubber was coated with paraffin. The rate of flow, initially measured with a calibrated flowmeter, was maintained nearly uniform throughout the experiment, by means of bubble count, at about 180 cc per minute, on each of the four absorbing chains. This adjustment was checked three times a week. The apparatus was placed in the basement, away from direct light and abrupt fluctuations in temperature.

ANALYTICAL METHODS

Analyses here reported were made in accordance with the procedures recommended by the Association of Official Agricultural Chemists (*2*), with the following exceptions: Total nitrogen was determined by a method combining the Devarda-alloy and the Gunning-Hibbard procedures (*5*); ammonium, by distillation in the presence of magnesium oxide (*1*); organic carbon, by a wet-combustion procedure;[4] and sodium, by weighing sodium uranyl zinc acetate hexahydrate (*3*).

The difference between the sums of nitrate, bicarbonate, sulphate, and chloride on the one hand, and of calcium, magnesium, potassium, sodium, and ammonium on the other, in no instance exceeded 0.5 percent of the total. In the case of the air-dried soils, the difference between the duplicate determinations of nitrogen (*2*) and carbon did not exceed 1 percent of each.

DESCRIPTION OF SOILS

The two sandy loam soils used in these studies were collected in the proximity of the Rubidoux Laboratory, Riverside, Calif. Both are transported soils, derived from the granites of Mount Rubidoux, and

[4] The writer is indebted to H. D. Chapman, of the University of California Citrus Experiment Station, and F. J. Foote, of the Limoneira Co. Laboratories, Santa Paula, Calif., for their modifications of the method.

both are quite similar in their characteristics, the only essential difference being that one has a much higher content of organic matter. One of these soils, designated "T," had received little or no fertilization for the past several years. The vegetative cover of this soil (trees) had been removed 1 year before the date of the collection of the sample, which was taken in January 1936 from the 6- to 12-inch horizon. The sample of the second soil, designated "F," was taken in March 1936 from the 12- to 24-inch horizon of an orange grove (plot F of the University of California Rubidoux fertilizer experiment), which had been manured repeatedly. The third soil, designated "F–Na," was a portion of soil F that had been leached with sodium chloride until about 0.9 of the original absorbed bases had been replaced by sodium. This sodium chloride-treated soil, F–Na, was rapidly dried under a fan, screened, and reserved for subsequent incubation tests.

The moisture equivalents of soils T, F, and F–Na were 11.0, 13.2, and 15.3, respectively; ammonium-absorbing capacities, 7.50, 7.87, and 8.02 milliequivalents per 100 g; total nitrogen, 6.4, 10.9, and 10.0 milligram atoms per 100 g; total carbon, 52, 126, and 122 mg atoms per 100 g. Soils F and T contained 5 and 15 milliequivalents of dissolved salts per kilogram of soil, chiefly bicarbonates, nitrates, sulphates, and chlorides of calcium and magnesium; while soil F–Na contained 60 milliequivalents of sodium chloride per kilogram and no other salts at zero time. The pH values of 1-to-1 aqueous extracts of soils F and T were 7.0 and 6.8 respectively, but no satisfactory determination of pH could be made for soil F–Na.

RESULTS

Tables 1 to 4 comprise data showing the results of the present investigation, obtained by following the foregoing experimental procedure. Concentrations of carbon and nitrogen in the soil extracts and in the air-dry soils are expressed as milligram atoms per kilogram. One milligram atom of carbon corresponds to 1 millimol of carbon dioxide, 1 milliequivalent of bicarbonate, or 12 mg of organic carbon. In a similar way, 1 mg atom of nitrogen corresponds to 1 milliequivalent of nitrate, 1 milliequivalent of ammonium, 1 millimol of ammonia, or 14 mg of nitrogen.

The findings reported in tables 1 to 4 represent primarily the effects of individual salts on individual soils under certain environmental conditions. They afford, nevertheless, a basis for some cautious generalizations. In table 5 the data are arranged with a view to facilitating such generalizations.

A preponderance of sodium salts in the soil moisture of incubated soils was found to be generally accompanied, with or without artificial aeration, by substantial increases in the amounts of nitrate and total nitrogen and of carbon dioxide and total carbon released by the soils in the course of several successive incubation extractions. The carbon-nitrogen ratios of the soils tended to become narrower as a consequence of the sodium treatment. This tendency, however, was obscured in the absence of aeration.

TABLE 1.—*Effect of sodium and calcium chlorides and sulphates on quantity of nitrogen and carbon released by aerated soils*

SOIL F

Electrolyte	Period of aeration [1]	Released per kilogram soil, oven-dry basis								
		Nitrogen					Carbon			
		Extracted			Volatilized ammonia	Total	Extracted		Volatilized carbon dioxide	Total
		Nitrate	Ammonium	Other forms [2]			Organic carbon	Bicarbonate		
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	6.22	0.14	0.15	0.05	6.56	19.8	4.4	13.9	38.1
	Second	2.48	0	.76	.37	3.61	1.0	3.2	26.3	30.5
	Third	.72	.47	.14	.37	1.70	5.6	2.3	22.6	30.5
Total		9.42	.61	1.05	.79	11.87	26.4	9.9	62.8	99.1
Sodium sulphate	First	5.82	.18	.48	0	6.48	15.9	3.7	26.1	45.7
	Second	3.70	0	0	0	3.70	4.8	1.6	24.0	30.4
	Third	1.35	0	.26	0	1.61	7.2	1.4	19.8	28.4
Total		10.87	.18	.74	0	11.79	27.9	6.7	69.9	104.5
Calcium chloride	First	5.39	.17	0	.11	5.67	8.3	2.6	15.4	26.3
	Second	2.43	0	.36	.28	3.07	.7	1.4	16.0	18.1
	Third	.79	0	.10	0	.89	2.2	.4	8.8	11.4
Total		8.61	.17	.46	.39	9.63	11.2	4.4	40.2	55.8
Calcium Sulphate	First	5.67	0	.24	0	5.81	1.9	2.1	25.2	29.2
	Second	1.61	0	0	0	1.61	2.7	.7	4.6	8.0
	Third	.89	0	.15	0	1.04	.5	1.1	12.0	13.6
Total		8.17	0	.39	0	8.56	5.1	3.9	41.8	50.8
Distilled water	First	5.39	.15	.53	.05	6.12	17.6	5.4	20.4	43.4
	Second	3.04	0	0	.23	3.27	.1	2.9	24.2	27.2
	Third	.42	0	.77	.41	1.80	3.5	1.0	8.3	12.8
Total		8.85	.15	1.30	.69	11.19	21.2	9.3	52.9	83.4

SOIL F-Na [3]

Sodium chloride	First	7.46	0.33	1.83	0	9.62	61.8	10.7	32.3	104.8
	Second	3.37	0	0	0	3.37	13.4	2.5	15.6	31.5
	Third	.62	0	.46	.02	1.10	2.5	.9	13.7	17.1
Total		11.45	.33	2.29	.02	14.09	77.7	14.1	61.6	153.4
Sodium sulphate	First	3.56	.43	3.79	0	7.78	57.7	11.1	33.0	101.8
	Second	3.16	0	0	.02	3.18	16.1	2.3	15.9	34.3
	Third	1.16	0	.63	.03	1.82	0	1.2	14.5	15.7
Total		7.88	.43	4.42	.05	12.78	73.8	14.6	63.4	151.8
Calcium chloride	First	5.13	.33	.58	.02	6.06	11.5	2.9	27.6	42.0
	Second	.74	0	.42	0	1.16	0	.2	11.9	12.1
	Third	1.28	0	0	0	1.28	0	.6	8.9	9.5
Total		7.15	.33	1.00	.02	8.50	11.5	3.7	48.4	63.6
Calcium sulphate	First	5.21	.41	.94	.01	6.57	14.8	5.0	29.1	48.9
	Second	1.31	0	71	0	2.02	0	.3	14.3	14.5
	Third	.49	0	.91	0	1.40	0	.6	10.9	11.5
Total		7.01	.41	2.56	.01	9.99	14.8	5.9	54.2	74.9

[1] Each experiment comprised 3 successive 15-day aeration periods, the first, second, and third being days 1–15, 16–30, and 31–45, respectively. Each period was preceded and followed by a 1-to-1 extraction with an electrolyte. Controls were extracted with distilled water.

[2] By difference: Total N minus NO_3, minus NH_4.

[3] With this soil, control extractions with distilled water were not technically feasible.

TABLE 1.—*Effect of sodium and calcium chlorides and sulphates on quantity of nitrogen and carbon released by aerated soils*—Continued

SOIL T

Electrolyte	Period of aeration	Released per kilogram soil, oven-dry basis								
		Nitrogen					Carbon			
		Extracted			Volatilized ammonia	Total	Extracted		Volatilized carbon dioxide	Total
		Nitrate	Ammonium	Other forms			Organic carbon	Bicarbonate		
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	9.65	0	0.61	0	10.26	8.6	2.4	15.4	26.4
	Second	.71	0	.14	0	.85	7.1	1.4	20.4	28.9
	Third	.89	0	.08	0	.97	4.9	1.6	21.4	14.9
Total		11.26	0	.83	0	12.08	20.6	5.4	57.2	70.2
Sodium sulphate	First	9.74	0	.17	0	9.91	6.7	2.7	16.9	26.3
	Second	1.10	0	0	0	1.10	4.8	.8	17.4	23.0
	Third	.32	0	.28	0	.60	4.5	1.2	7.7	13.4
Total		11.16	0	.45	0	11.61	16.0	4.7	42.0	62.7
Calcium chloride	First	9.32	0	.36	0	9.68	7.2	2.0	12.8	22.0
	Second	.64	0	.01	0	.68	.2	1.0	18.0	19.2
	Third	.96	0	0	0	.96	1.4	1.0	12.8	15.2
Total		10.92	0	.40	0	11.32	8.8	4.9	43.6	56.4
Calcium sulphate	First	7.26	0	.30	0	7.56	3.1	2.0	15.0	20.1
	Second	.91	0	0	0	.91	1.8	.6	10.2	12.6
	Third	.53	0	.18	0	.71	.9	.9	6.8	8.6
Total		8.70	0	.48	0	9.18	5.8	3.5	32.0	41.3
Distilled water	First	9.30	0	.33	0	9.63	9.4	3.5	13.4	26.3
	Second	1.08	0	.15	0	1.23	1.3	1.5	19.6	22.4
	Third	1.34	0	.03	0	1.37	3.2	1.0	13.8	18.0
Total		11.72	0	.51	0	12.23	13.9	6.0	46.8	66.7

Comparisons made in table 5 suggest the term "stimulation" as descriptive of the effect of sodium salts on the transformations of carbon and nitrogen in the soils under the experimental conditions previously outlined. The stimulating effect of the sodium salts is manifested in the increased quantities of nitrate, total nitrogen, carbon dioxide, and the soluble forms of carbon that were released in the course of the incubation-extraction treatments (tables 1 and 2). While the differences between the sodium-treated soils and the distilled-water series were not so great as the differences between the sodium-treated and the calcium-treated soils, they were significant in three cases out of four. There was no appreciable difference between the effects of sodium chloride and of sodium sulphate on the transformations of the organic matter in the soil, despite one rather striking exception (table 2). The differences between calcium chloride and calcium sulphate, however, were conspicuous and irregular. In contrast with the observations of Greaves et al. (*11*), calcium sulphate was found to be more often a deterrent than a stimulant to nitrification. The stimulating effects of the sodium salts previously shown were emphasized in consequence of the presaturation of the soil with sodium (soil F–Na). The striking increase

in the production of nitrate parallels, in some respects, the experience of Greaves (*10*) with leached alkali soils. The failure of the ionic antagonism to manifest itself in the sulphate-treated soil F–Na is rather surprising and cannot be harmonized with the results of Lipman (*14*).

TABLE 2.—*Effect of sodium and calcium chlorides and sulphates on the quantity of nitrogen and carbon released by soils in the absence of aeration*

Electrolyte	Period of incubation[1]	Released per kilogram of soil, oven-dry basis						
		Nitrogen				Carbon		
		Extracted			Total	Extracted		Total
		Nitrate	Ammonium	Other forms[2]		Organic carbon	Bicarbonate	
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
SOIL F								
Sodium chloride	First	6.23	0	0.57	6.80	0	1.3	1.3
	Second	1.17	0	.89	2.06	4.0	2.1	6.1
	Third	1.36	0	.61	1.97	0	1.4	1.4
Total		8.76	0	2.07	10.83	4.0	4.8	8.8
Sodium sulphate	First	7.06	0	.12	7.18	0	2.4	2.4
	Second	0	.41	.68	1.09	7.7	9.0	16.7
	Third	2.55	0	0	2.55	0	1.6	1.6
Total		9.64	.41	.80	10.82	7.7	13.0	20.7
Calcium chloride	First	6.61	0	.14	6.75	0	1.0	1.0
	Second	.21	0	.52	.73	.3	.6	.9
	Third	1.19	0	0	1.19	0	.8	.8
Total		8.01	0	.66	8.67	.3	2.4	2.7
Calcium sulphate	First	.17	1.42	.68	2.27	0	11.1	11.1
	Second	0	.64	.93	1.57	.3	6.7	7.0
	Third	1.85	0	0	1.85	0	1.6	1.6
Total		2.02	2.06	1.61	5.69	.3	19.4	19.7
Distilled water	First	7.04	0	0	7.04	0	1.2	1.2
	Second	1.30	0	.53	1.83	.9	2.8	3.7
	Third	1.05	0	.12	1.17	0	1.1	1.1
Total		9.39	0	.65	10.04	.9	5.1	6.0
SOIL F–Na[3]								
Sodium chloride	First	4.32	0.41	1.50	6.23	32.3	3.6	35.9
	Second	1.44	.01	2.78	4.23	57.1	5.2	62.3
	Third	0	.86	.27	1.13	31.0	0	31.0
Total		5.76	1.28	4.55	11.59	120.4	8.8	129.2
Sodium sulphate	First	4.32	.83	1.22	6.37	39.9	3.6	43.5
	Second	0	.15	2.59	2.74	65.0	6.5	71.5
	Third	.20	1.12	1.02	2.34	30.3	0	30.3
Total		4.52	2.10	4.83	11.45	135.2	10.1	145.3
Calcium chloride	First	1.44	.28	.50	2.22	14.1	.8	14.9
	Second	.83	.16	.34	1.33	12.6	1.3	13.9
	Third	1.11	.49	0	1.60	0	.5	.5
Total		3.38	.93	.84	5.15	26.7	2.6	29.3
Calcium sulphate	First	3.46	.41	.98	4.85	21.8	1.7	23.5
	Second	.11	.38	.48	.97	2.5	1.6	4.1
	Third	0	.52	.11	.63	2.4	.1	2.5
Total		3.67	1.31	1.57	6.45	26.7	3.4	30.1

[1] The first, second, and third 15- or 30-day periods of incubation included days 1–30, 30–45, and 45–60 respectively for soil F; days 1–15, 16–30, 31–45 respectively for soil F–Na; and days 1–15, 16–30, and 31–60 respectively for soil T. Each period was preceded and followed by a 1-to-1 extraction with an electrolyte. Controls were extracted with distilled water.

[2] By difference: Total N minus NO_3, minus NH_4.

[3] With this soil, control extractions with distilled water were not technically feasible.

TABLE 2.—*Effect of sodium and calcium chlorides and sulphates on the quantity of nitrogen and carbon released by soils in the absence of aeration*—Continued

SOIL T

Electrolyte	Period of incubation	Released per kilogram of soil, oven-dry basis						
		Nitrogen				Carbon		
		Extracted			Total	Extracted		Total
		Nitrate	Ammonium	Other forms		Organic carbon	Bicarbonate	
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
Sodium chloride	First	9.58	0.06	0.25	9.89	9.1	3.2	12.3
	Second	0	.03	0	.03	6.0	2.4	8.4
	Third	2.87	0	1.13	4.0	3.7	1.7	5.4
Total		12.45	.09	1.38	13.92	18.8	7.3	26.1
Sodium sulphate	First	8.99	.07	.74	9.80	7.9	2.4	10.3
	Second	0	.01	0	.01	5.1	2.0	7.1
	Third	.29	.12	.91	1.32	7.8	4.9	12.7
Total		9.28	.20	1.65	11.13	20.8	9.3	30.1
Calcium chloride	First	7.92	.26	.48	8.66	6.1	3.9	10.0
	Second	0	.01	0	.01	4.4	.4	4.8
	Third	1.70	.17	.23	2.10	1.9	1.1	3.0
Total		9.62	.44	.71	10.77	12.4	5.4	17.8
Calcium sulphate	First	8.23	.16	.55	8.94	6.5	1.7	8.2
	Second	.35	.14	0	.49	3.0	1.4	4.4
	Third	1.76	.03	.32	2.11	3.5	1.2	4.7
Total		10.34	.33	.87	11.54	13.0	4.3	17.3
Distilled water	First	7.87	.02	.35	8.24	6.5	4.3	10.8
	Second	0	.07	0	.07	3.2	.9	4.1
	Third	2.28	0	0	2.28	4.1	1.7	5.8
Total		10.15	.09	.35	10.59	13.8	6.9	20.7

An attempt to explain the stimulating effect of the sodium salts upon nitrification and the production of carbon dioxide, and the increase in the amounts of soluble nitrogen and carbon released under the influence of the sodium salts, leads, in the present state of our knowledge, to the following considerations:

The greater solubility of the organic matter of the soil in the presence of the sodium salts is indicated by the consistently larger quantities of the organic carbon that were present in the extracts of the sodium-treated soils. An increase in the solubility of the organic matter may in itself prove to be a stimulant to the microbiological processes in the soil under favorable environmental conditions. This stimulation may be due to a simple increase in the available amounts of substances needed for the development of the soil microflora, to a possible increase in the amounts of soluble phosphate in the presence of dissolved organic matter, or to some complicated and obscure influence that may be designated as the "specific" effect of the dissolved organic matter. A remarkable increase in the density of bacterial population observed by Chizhévsky (*4*) as a consequence of the saturation of the soil with sodium can be attributed either to the effect of absorbed sodium on the environment or to the increase in the soluble organic matter or to both.

TABLE 3.—*Total nitrogen and carbon in air-dry soils at the beginning and at the end of the tests*

Soil	Electrolyte	Aerated soils								Unaerated soils							
		Nitrogen per 100 g				Carbon per 100 g				Nitrogen per 100 g				Carbon per 100 g			
		Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment	Initial total	Final total	Difference	Removed during experiment
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
F	Sodium chloride	10.9	11.4	+0.5	1.2	136	115	−21	10	10.9	9.9	−1.0	1.1	136	105	−31	1
	Sodium sulphate		10.5	−.4	1.2		108	−28	10		9.8	−1.1	1.1		105	−31	2
	Calcium chloride		11.0	+.1	1.0		122	−14	6		10.3	−.6	.9		130	−6	1
	Calcium sulphate		10.0	−.9	.9		118	−18	5		10.3	−.6	.6		118	−18	2
	Distilled water		10.1	−.8	1.1		114	−22	8		9.3	−1.6	1.0		111	−25	1
F–Na [1]	Sodium chloride	10.1	8.1	−2.0	1.4	122	93	−29	14	10.0	8.1	−1.9	1.2	122	105	−17	13
	Sodium sulphate		7.4	−2.7	1.3		95	−27	12		8.3	−1.7	1.1		105	−17	15
	Calcium chloride		8.3	−1.8	.9		106	−16	10		8.8	−1.2	.5		112	−10	3
	Calcium sulphate		9.5	−.6	1.0		105	−17	10		8.6	−1.4	.6		110	−12	3
T	Sodium chloride	6.4	4.7	−1.7	1.2	52	46	−6	8	6.4	4.5	−1.9	1.4	52	46	−6	3
	Sodium sulphate		5.2	−1.2	1.2		47	−5	6		4.4	−2.0	1.1		50	−2	3
	Calcium chloride		4.4	−2.0	1.1		49	−3	6		4.3	−2.1	1.1		48	−4	2
	Calcium sulphate		4.5	−1.9	.9		47	−5	4		4.3	−2.1	1.1		49	−3	2
	Distilled water		4.5	−1.9	1.2		46	−6	7		4.3	−2.1	1.0		50	−2	2

[1] Extraction of this soil with distilled water was not technically feasible.

TABLE 4.—*Carbon-nitrogen ratios of air-dry soils at the beginning and at the end of the tests*

Soil	Electrolyte	Ratio of total carbon to total nitrogen in—			
		Aerated soil		Unaerated soil	
		Initial	Final	Initial	Final
		Milligram atoms	*Milligram atoms*	*Milligram atoms*	*Milligram atoms*
F	Sodium chloride	12.5	10.1	12.5	10.6
	Sodium sulphate		10.3		10.7
	Calcium chloride		11.1		12.6
	Calcium sulphate		11.8		11.5
	Distilled water		12.2		11.9
F-Na[1]	Sodium chloride	12.2	11.5	12.2	13.0
	Sodium sulphate		12.8		12.6
	Calcium chloride		12.8		12.7
	Calcium sulphate		11.1		12.8
T	Sodium chloride	8.2	9.7	8.2	10.2
	Sodium sulphate		9.0		11.3
	Calcium chloride		11.1		11.2
	Calcium sulphate		10.5		11.3
	Distilled water		10.2		11.6

[1] Extraction of this soil with distilled water was not technically feasible.

TABLE 5.—*Total nitrogen and total carbon released by soils, expressed as percentages of the calcium chloride-treated series*

Electrolyte	Total nitrogen in—						Total carbon in—					
	Aerated soil			Unaerated soil			Aerated soil			Unaerated soil		
	F	F-Na	T	F	F-Na	T	F	F-Na	T	F	F-Na	T
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Sodium chloride	123	166	107	125	225	129	178	241	136	326	441	147
Sodium sulphate	122	150	103	125	222	103	187	239	112	767	496	169
Calcium chloride	100	100	100	100	100	100	100	100	100	100	100	100
Calcium sulphate	89	118	81	66	125	107	91	118	73	730	103	97
Distilled water	114	(1)	108	116	(1)	98	149	(1)	118	222	(1)	116

[1] Extraction of this soil with distilled water was not technically feasible.

The greater solubility of the organic matter in the sodium-treated soils cannot be understood so easily as some superficial considerations would lead us to believe. Neither the percentage of sodium in the soil extracts, nor the pH trends, nor the amounts of carbon dioxide released, nor the amounts of nitrogen nitrified can be related directly to the amounts of humus extracted at any given time. Nor is there any significant parallelism between any two of the above-enumerated quantities insofar as the rates of their release are concerned. The total amounts of carbon, however, released from period to period (1–15 days, 16–30 days, etc.) do parallel the total amounts of nitrogen released during the same period, in the sense that the greater amounts of one frequently correspond to the greater amounts of the other. The grand total amounts of nitrogen and carbon, on the other hand, representing the cumulative effects of the entire experiment, suggest the possibility of correlation, if not of proportionality. It may be observed here that the carbon-nitrogen ratio of the extracts of all

sodium-treated soils is substantially higher than it is for the corresponding calcium-treated soils.

The amounts of nitrogen released by the soils invariably decreased from period to period. The bulk of nitrate and of the total nitrogen was, as a rule, extracted after the first 15 days of the incubation. There was no such abrupt change in the production of carbon dioxide, however, while the amounts of the soluble carbon, after a drop between the fifteenth and the thirtieth day of the incubation, would often increase during the next 15-day period. The stimulating effect of the sodium salts was repeatedly manifested throughout the entire range of these fluctuations, although the degree of stimulation varied considerably from time to time and from soil to soil.

The fluctuations in the rate of the transformations of organic matter in the soils suggest, perhaps, a degree of periodicity in the activities of the different groups of the soil micro-organisms. The reactions of the carbon and nitrogen cycles in the soil, though interdependent, may not be entirely contemporaneous. Moreover, as the relative proportions of the inorganic constituents in the soil moisture are altered, in the course of the present experiment, in the direction of a complete proponderance of either sodium or calcium salts, the possibilities of the ionic antagonism (*14*) become minimized.

The release of nitrogen by the soils was stimulated to the extent of 7 to 70 percent in the presence of sodium salts if the comparison be made on the basis of the corresponding and parallel calcium-treated series. A far greater stimulation was observed in the transformations of the soil carbons. It would seem reasonable therefore that the sodium-treated soils, having lost relatively more carbon than nitrogen in the course of the experiment, would be left with a narrower C : N ratio than they had heretofore possessed. This, however, was not always the case (table 4).

There were unaccounted-for gains in nitrogen, suggestive of nitrogen fixation, in soil F. The unaccounted-for losses of both carbon and nitrogen (table 3), observed in the majority of cases, had probably taken place during the 6 weeks' drying in the air that followed the incubation-extraction treatments, and, perhaps, during the extraction periods. Significant as these losses are, they scarcely invalidate the measurements of either the released or the residual amounts. The C : N ratio of soil F tended to become narrower, regardless of treatment. This tendency, however, was much more emphatic in the presence of sodium salts than in the presence of calcium salts. Soil T, on the other hand, tended to increase its C : N ratio in all instances. Here, again, the narrower ratio was found in the case of the sodium-treated soils. It appears, accordingly, that, at least in this particular instance, sodium salts exert an influence of their own on the C : N ratio of the soil.

Among minor observations incidental to the main purpose of the experiment, the effect of sulphate on the unaerated soil F is to be noted. Here the preponderance of gypsum, as well as of sodium sulphate, though to a lesser extent, was soon accompanied by the appearance of large amounts of bicarbonate and ammonia and by the odor suggestive of the reduction of the sulphates. After 30 days nitrate disappeared from the soil solution. It is of interest that the 30-day extract of the gypsum-treated soil attains the threshold of phenol-

phthalein alkalinity. It is likewise of interest that the reducing properties of sulphate-treated soils apparently lost some of their intensity some time after the 45 days' incubation. No such effects were observed in the other two soils, although the nitrification apparently ceased in both (with the exception of the calcium chloride-treated F–Na and the calcium sulphate-treated T) after 15 to 30 days' incubation.

The release and volatilization of ammonia by the aerated soil F can hardly be harmonized either with characteristics of the medium (such as its aerobic character and only faintly alkaline pH) or with the opinion that the release of ammonia in the soils is one of the consequences of the narrowing of their C : N ratio (*17*). One may suppose that, in this particular soil, ammonia had been removed by the stream of air before it could be nitrified, on the general assumption that ammonification normally preceded nitrification.

CONCLUSIONS

Conservation of soil organic matter in the form of stable humus reserves is regarded by agronomists as an important aspect of the major problem of maintaining the productivity of soils. It has been recognized in this connection that sodium, absorbed or present in the soil solution, tends to promote depletion of organic reserves of the soil (*9*). Emphasis heretofore has been placed on sodium primarily in connection with high alkalinity. The results of the present investigation indicate that neutral salts of sodium, even in concentrations sufficient to prevent deflocculation of the soil, dissolve significant quantities of organic matter. The presence of neutral salts in soils can be regarded, accordingly, as a factor unfavorable to the conservation of organic matter.

The stimulating effect of sodium salts on mineralization of nitrogen and, perhaps, on the solubility of other nutritive substances of the soil, has agronomic consequences and invokes important nutritional considerations. To what extent the beneficial effects that sometimes have been observed to follow the application of sodium chloride to lands can be attributed to the sodium effect as distinct from the chloride effect cannot now be stated. The results here presented suggest, however, that in many instances the observed responses have occurred at the expense of the organic reserves of the soil.

SUMMARY

The effect of sulphates and chlorides of sodium and calcium on the microbiological transformations of the organic matter in two sandy loam soils was studied by determining volatile and soluble products of bacterial action that were released by aerated soils in the course of three successive 15-day incubation periods, each preceded and followed by a 1-to-1 extraction with N/20 solutions of the indicated electrolytes. Parallel series of tests were conducted with the same soils incubated in the absence of artificial aeration. The experimental results suggested the following conclusions:

(1) The release of nitrate and of total nitrogen, as well as of the soluble and volatile forms of carbon, is stimulated, often to a great

extent, by the sodium salts and depressed by the corresponding calcium salts, regardless of aeration.

(2) Presaturation of the soil with sodium tends to emphasize the stimulating effects of the sodium salts.

(3) The carbon-nitrogen ratio of the undissolved organic matter of the soil is lower in the sodium-treated than in the calcium-treated, aerated soils.

Without attempting to explain the mechanism of the stimulating effects of sodium salts, the author discusses these effects with reference to the influence of sodium on the soil organic matter and to the possible physiological influences of the soluble organic matter, as well as of the electrolytes themselves, on the activities of the organisms responsible for the carbon and nitrogen transformations in the soil.

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AN APPARENT INDUCED LOSS OF NITROGEN-FIXING ABILITY IN AZOTOBACTER [1]

By CHARLES R. STUMBO, formerly *graduate assistant in bacteriology*, and P. L. GAINEY, *soil bacteriologist, Kansas Agricultural Experiment Station*

INTRODUCTION

There is abundant evidence that *Azotobacter* will not make use of free atmospheric nitrogen in its metabolism if it has at its disposal an adequate quantity of available combined nitrogen. Such being the case, it does not seem unreasonable to suppose that if cultures of the organism were grown under conditions where the utilization of atmospheric nitrogen was completely suppressed for a sufficient period the organism might lose its ability to metabolize free nitrogen. Such an alteration in the metabolism of *Azotobacter* would be of interest from a practical as well as from a theoretical point of view since in fertile soils a concentration of available nitrogen sufficient to inhibit fixation is frequently present for considerable periods of time. Briscoe [2] has recorded a limited amount of data indicating that some such change does take place; she found that after prolonged growth on media high in nitrate nitrogen some strains of *Azotobacter* did not grow normally when transferred to a medium low in fixed nitrogen. The primary objectives of the present investigation were to verify Briscoe's work and to ascertain whether the failure of *Azotobacter* to grow normally on a nitrogen-low medium after culture at high concentrations of nitrate nitrogen could be attributed to a loss of its ability to metabolize free nitrogen.

PROCEDURE

Pure cultures of *Azotobacter chroöcoccum* (all strains studied produced a black pigment and were arbitrarily classed as *chroöcoccum*) were isolated from local soils and grown both on nitrogen-free [3] mannite agar and on the same medium containing various concentrations of nitrate nitrogen as potassium nitrate. Transfers were made every 3 or 4 days and incubation was at 28° to 30° C. In this way it was possible to determine the ability of any particular strain to tolerate nitrate nitrogen. After various intervals, transfers were made from the highest concentrations of nitrate nitrogen agar upon which normal (macroscopically equal to growth on nitrogen-free agar) growth took place to nitrogen-free agar, and to agar containing very low concentrations of nitrate nitrogen. By comparing the growth of the various strains on (1) the high nitrate nitrogen agar, (2) the high nitrate nitrogen agar cultures transferred to nitrogen-free agar, and (3) the

[1] Received for publication December 31, 1937; issued August 1938.

[2] BRISCOE, FAITH WINIFRED. THE EFFECT OF AVAILABLE NITROGEN UPON THE GROWTH AND NITROGEN-FIXING ABILITY OF AZOTOBACTER. Master Sci. Thesis, Kansas State College Library. 1933.

Contribution No. 171, Department of Bacteriology, Kansas Agricultural Experiment Station.

[3] "Nitrogen-free" as used in this paper refers to agar that contained no more than 5 to 10 p. p. m. of combined nitrogen.

same strain grown continuously on nitrogen-free agar, it was possible to note any marked change in the growth of a given strain on nitrogen-free agar resulting from previous prolonged growth under conditions where nitrogen fixation was suppressed. More than 150 strains were studied in this way.

PRELIMINARY DATA

In a recent paper from this laboratory [4] data were submitted relative to the tolerance of *Azotobacter chroöcoccum* to nitrate nitrogen. These data indicated considerable strain variation in this respect, but most of the strains tested grew readily on mannite agar containing 3,000 to 4,000 parts per million of nitrate nitrogen. Additional strains have been tested and the results previously reported are amply verified by the data obtained.

Attention was also called in the above-mentioned paper to an apparently greater toxicity of the nitrate ion when cultures were transferred at infrequent intervals, a number of cultures having been accidentally lost by failure to transfer at the regular 3- to 4-day interval. Additional information was sought on this point and sufficient data relative thereto are on record to prove that the earlier observations were correct.

In table 1 are presented data showing the concentrations of nitrate nitrogen tolerated by one series of 46 freshly isolated and 3 previously isolated strains, together with records of growth of each strain on nitrogen-free and on 100 p. p. m. nitrate nitrogen agar, after having been grown on the high nitrate nitrogen agar for 105 days. The 1,000, 3,000, and 4,000 p. p. m. of nitrate nitrogen were the highest concentrations tested upon which the various strains listed thereunder would consistently produce normal growth. The term "normal" as here used merely means that the growth appeared macroscopically to equal that of the same culture growing continuously on the nitrogen-free agar. The concentration of 100 p. p. m. of nitrate nitrogen agar was employed because previously reported investigations (Burk et al.) [5] indicated that this was approximately the minimum concentration that would supply adequate nitrogen for maximum growth without the assimilation of atmospheric nitrogen.

In subsequent discussions the cultures that were kept continuously on nitrogen-free agar will be referred to as the 0 cultures, while those maintained on 1,000, 3,000, 4,000, etc., p. p. m. of nitrate nitrogen agar will be referred to as the 1,000 p. p. m., 3,000 p. p. m., 4,000 p. p. m., etc., cultures.

From the data presented in table 1 it is evident that all 1,000, 3,000, and 4,000 p. p. m. cultures grew readily when transferred to 100 p. p. m. nitrate nitrogen agar, but that 18 of the 49 cultures tested in this instance failed to grow or grew very poorly on the nitrogen-free agar. The difference in the growth of strain 4,000 p. p. m. O–2 and 0 O–2 upon a nitrogen-free agar is illustrated in figure 1. The number of strains growing abnormally on nitrogen-free agar, following growth on high nitrate nitrogen agar, was considerably larger than noted in previous tests. In view of the fact that the so-called nitrogen-free agar actually contained from 5 to 10

[4] GAINEY, P. L. THE TOLERANCE OF NITRATE BY PURE CULTURES OF AZOTOBACTER. Soil Sci. 42: 445–459. 1936.

[5] BURK, DEAN, LINEWEAVER, HANS, and HORNER, C. KENNETH. THE SPECIFIC INFLUENCE OF ACIDITY ON THE MECHANISM OF NITROGEN FIXATION BY AZOTOBACTER. Jour. Bact. 27: 325–340, illus. 1934.

p. p. m. of nondializable nitrogen, it was thought that the limited growth observed might have resulted from the utilization of this nitrogen.

TABLE 1.—*Relative growth of 49 cultures of Azotobacter in media containing 0 and 100 p. p. m. of nitrate nitrogen following 105 days' growth at higher concentrations of nitrate nitrogen*

1,000 p. p. m.[1]			3,000 p. p. m.[1]			4,000 p. p. m.[1]		
Culture	Growth on nitrogen-free agar [2]	Growth at 100 p. p. m. nitrate nitrogen	Culture	Growth on nitrogen-free agar	Growth at 100 p. p. m. nitrate nitrogen	Culture	Growth on nitrogen-free agar	Growth at 100 p. p. m. nitrate nitrogen
K-1	4	4	E-3	3	3	D-3	3	3
K-2	4	4	C-4	4	4	I-1	3	3
H-1	4	4	J-6	4	4	I-2	?	3
F-1	4	4	O-3	3	3	I-3	3	3
J-2	?	3	1	?	3	I-4	4	4
B-1	?	3	4	?	2	I-5	3	3
B-3	?	4	6a [3]	?	3	I-6	3	3
B-4	4	4	E-1	3	3	J-5	4	4
H-3	4	4	G-2	4	4	N-2	3	3
A-4	?	4	G-3	4	4	N-1	4	4
F-2	?	3	G-1	4	4	N-4	?	2
C-1	4	4	H-2	3	3	O-2	?	3
C-2	?	3	J-1	?	3	P-1	?	2
C-3	4	4	J-3	3	3	P-3	?	3
N-3	1	1	L-1	3	3	5b [3]	?	3
0	4	4	N-5	4	4			
L-2	?	4						
5a [3]	?	4						

[1] Concentration of nitrate nitrogen at which *Azotobacter* was cultured for 105 days.
[2] ? indicates slight or no visible growth after 3 days; 1, 2, 3, and 4 indicate increasing quantities of growth, 4 representing normal growth of the strain in question when cultured on nitrogen-free agar continuously.
[3] Cultures grown at higher concentration for approximately 7 months.

It is obvious from these results that some change had taken place in the physiological make-up of certain strains of *Azotobacter* as a result of their being cultured on an agar medium of high nitrate nitrogen content. Subsequent tests proved that if the quantity of nitrate nitrogen furnished the 18 "abnormal" strains was inadequate to supply the needs of the organism, growth of young cultures was more or less proportional to the available nitrogen, whereas, growth of the other 31 strains was independent of the fixed nitrogen supplied. The most obvious inference to be drawn from these results is that the abnormal strains had partly or completely lost their ability to utilize atmospheric nitrogen. Definite proof of such an alteration, is, however, not so easily obtained.

GROWTH AND NITROGEN FIXATION ON AGAR

Quantitative methods were resorted to in an effort to ascertain whether the very limited growth produced by the 18 abnormal cultures on nitrogen-free agar was due to the assimilation of the 5 to 10 p. p. m. of fixed nitrogen present in the medium and whether the treatment to which the cultures had been subjected had altered their ability to utilize free nitrogen.

Fifty cubic centimeters of nitrogen-free or low nitrate nitrogen mannite agar were placed in large sterile Petri dishes (5½ inches in diameter). These were inoculated in quadruplicate with 0.3 cc of a saline suspension (roughly standardized to McFarland's nephelometer tube No. 2) of 48-hour agar slant cultures. The contents of two of the dishes were immediately analyzed for total nitrogen while the other two were incubated at 28° to 30° C. for 3 to 4 days, after which their total nitrogen content was determined. The data secured from three such

FIGURE 1.—Forty-eight-hour-old cultures of *Azotobacter* on nitrogen free agar: *a*, Culture of strain O–2 grown continuously on nitrogen free agar following isolation; *b*, the same strain as in *a* with several months preliminary culturing upon 4,000 p. p. m. nitrate-nitrogen agar.

tests with culture 4,000 p. p. m. 5b, three with culture 4,000 I–2, and one with culture 4,000 O–2 are recorded in tables 2 and 3. In parallel columns are indicated the mean relative growth, mean total nitrogen utilized, and mean atmospheric nitrogen fixed. The quantity of free atmospheric nitrogen metabolized is the factor of major importance, and the significance of the values recorded have been determined statistically according to Fisher's [6] method where limited replications are available. The significance of the mean values for nitrogen fixed is recorded in terms of P, where P represents the probability of a value equal to that recorded being due to chance. For example, if for the recorded mean difference in nitrogen content between the

[6] FISHER, RONALD AYLMER, STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London. 1934.

controls and treated samples $P=0.4$, then the probability is that 4 times out of 10 a value equal to that recorded will be due to chance alone. Differences for which the value P is greater than 0.05 are not regarded as significant.

TABLE 2.—*Growth and nitrogen fixed by cultures 5b and I–2, on agar varying in nitrogen content, with an incubation period of 3 days*

CULTURE 5b

Experiment No. and calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]				0 culture [2]			
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth	Total organic nitrogen per 50 cc media after growth	Nitrogen fixed per 50 cc media	P	Observed growth	Total organic nitrogen per 50 cc media after growth	Nitrogen fixed per 50 cc of media	P
Experiment 1:	*Mg*	*Mg*		*Mg*	*Mg*			*Mg*	*Mg*	
0	0	0.45	[3] ?	0.50	0.05	0.4	4	3.39	2.84	0.01
50	2.39	2.84	2	3.23	.39	.8	4	5.39	2.55	.01
100	4.80	5.25	3	6.20	.95	.01	4	6.50	1.25	.01
150	7.22	7.67	4	8.21	.54	.3	4	9.00	1.33	.01
Experiment 2:										
0	0	.48	1	1.26	.78	.01	4	4.41	3.93	.01
50	2.49	2.97	3	6.17	3.20	.01	4	6.34	3.39	.01
100	5.01	5.74	4	7.29	1.55	.01	4	7.71	1.97	.01
Experiment 3:										
0	0	.40	?	.56	.16	.2	4	5.61	5.21	.01
10	.51	.91	1	1.26	.35	.05				
25	1.36	1.76	2	2.51	1.15	.01				
50	2.62	3.02	3	4.07	1.05	.01				
100	5.46	5.86	4	6.75	.89	.01				
150	7.74	8.14	4	9.05	.92	.01				

CULTURE I-2

Experiment 1:										
0	0	0.45	1	1.29	0.84	0.01	4	6.35	5.90	0.01
50	2.39	2.84	4	4.31	1.47	.01	4	5.94	3.10	.01
100	4.80	5.25	4	6.66	1.41	.01	4	7.08	1.83	.01
150	7.22	7.67	4	8.03	.36	.05	4	9.00	1.33	.01
Experiment 2:										
0	0	.48	1	2.07	1.59	.01	4	8.34	7.86	.01
50	2.49	2.97	3	5.33	2.36	.01	4	7.01	4.04	.01
100	5.01	5.73	4	6.93	1.20	.01	4	7.50	1.77	.01
Experiment 3:										
0	0	.40	1	.84	.44	.01	4	10.02	9.62	.01
10	.51	.91	2	1.47	.56	.01				
25	1.36	1.76	3	3.12	1.36	.01				
50	2.62	3.02	4	5.55	2.53	.01				
100	5.46	5.86	4	8.62	2.76	.01				
150	7.74	8.14	4	10.31	2.17	.01				

[1] Previously grown at 4,000 p. p. m. nitrate nitrogen. See text for meaning of P.
[2] Previously grown on "nitrogen-free" agar.
[3] See footnote 2, table 1.

The quantity of nitrogen fixed was obtained by subtracting the total quantity of nitrogen originally present in the medium from that found at the end of the incubation period. This procedure seemed justifiable since in every instance except one qualitative tests for nitrate nitrogen, as well as the total quantity of organic nitrogen present, indicated that all nitrate nitrogen had been used up by the organisms.

The data presented in tables 2 and 3 indicate quite definitely certain facts, among which the following are of significance in this connection:

(1) The 0 culture of all three strains is capable of readily utilizing either free atmospheric or nitrate nitrogen.

TABLE 3.—*Growth and nitrogen fixed by culture O–2 on agar varying in nitrogen content, with incubation periods of 5 and 8 days*

Calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]							
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth after—		Total organic nitrogen per 50 cc media after growth for—		Nitrogen fixed per 50 cc of media			
			5 days	8 days	5 days	8 days	5 days	*P*	8 days	*P*
	Mg	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.24	[2] 1	1	0.28	0.28	0.04	0.2	0.04	0.2
2½	.12	.36	[2] 1	2	.48	.63	.12	.02	.27	.01
7½	.37	.61	[2] 1	2	.49	1.11	−.12		.50	.01

Calculated nitrate nitrogen added to media (p. p. m.)	Controls		0 culture [3]							
	Nitrate nitrogen per 50 cc media as determined	Total nitrogen per 50 cc media as determined	Observed growth after—		Total organic nitrogen per 50 cc media after growth for—		Nitrogen fixed per 50 cc of media			
			5 days	8 days	5 days	8 days	5 days	*P*	8 days	*P*
	Mg	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.24	4	4	5.54	5.76	5.45	0.01	5.67	0.01
2½	.12	.36								
7½	.37	.61								

[1] Previously grown at 4,000 p. p. m. of nitrate nitrogen. See text for meaning of *P*.
[2] See footnote 2, table 1.
[3] Previously grown on "nitrogen-free" agar.

(2) In the presence of less nitrate nitrogen than the culture is capable of making use of, the quantity of atmospheric nitrogen fixed decreases as the quantity of nitrate nitrogen increases. A concentration of 150 p. p. m. of nitrate nitrogen was inadequate to completely inhibit fixation in the few instances tested.

(3) Fixation of nitrogen by the 4,000 p. p. m. cultures on nitrogen-free agar occurred in the case of I–2 (three trials), did not take place in the case of O–2 (one trial), and was significant in only one out of three trials in the case of 5b.

(4) Fixation of nitrogen by the 4,000 p. p. m. cultures was stimulated in the case of all three strains by the addition of small quantities of nitrate nitrogen.

(5) Where fixation of atmospheric nitrogen was accomplished by a 4,000 p. p. m. culture on nitrogen-free agar, the quantities fixed were nearly always very small as compared with the quantities fixed by the O culture under identical conditions.

From these results it appears that the previous treatment of the 4,000 p. p. m. cultures of the three strains studied quantitatively had effected a radical alteration in the ability of the organism to metabolize free atmospheric nitrogen.

GROWTH AND NITROGEN FIXATION IN LIQUID MEDIA

Since the presence in the agar medium of limited quantities of nitrate nitrogen, even as small as 2½ p. p. m., stimulated fixation of

nitrogen by the 4,000 p. p. m. cultures, and the so-called nitrogen-free agar contained from 5 to 10 p. p. m. of combined nitrogen, it was thought that this nondializable nitrogen, present in the agar-agar, possibly might have acted as a stimulant to fixation in those instances in which fixation was noted on the nitrogen-free medium. It was, therefore, deemed advisable to undertake similar studies with a liquid medium in which the concentration of fixed nitrogen could be reduced much lower, i. e., 0.9 to 1.5 p. p. m. The same medium without the addition of the agar was employed.

Growth of *Azotobacter* in a stationary liquid medium takes place very slowly unless the layer of the liquid is extremely thin. In order to make use of sufficient media for quantitative purposes without employing relatively large cultural vessels it was necessary to resort to aeration of cultures as suggested by Hunter [7] for increasing the rate of growth.

One hundred cubic centimeters of the medium were placed in 300-cc pyrex Erlenmeyer flasks, fitted with an aeration tube inserted in a cork stopper well wrapped with cotton, and the flask with its contents was sterilized. The aeration tube was prepared by sealing the end of a piece of ordinary glass tubing and perforating it laterally a number of times near the sealed end, to facilitate distribution of the air.

Air under pressure was passed successively through similar aeration tubes inserted respectively in a bottle of weak sulphuric acid, a bottle of weak sodium hydroxide, a bottle of weak mercuric chloride, and two bottles of distilled water. The chief purpose in using the water was to saturate the air with moisture in order to reduce to a minimum the loss of the culture medium through evaporation.

The flasks were inoculated in replications of two to five (usually five) with one drop of a suspension of the organisms prepared as previously described. The quantity of nitrogen contained in one drop of the inoculum was so small that it could be ignored, hence the aerated controls were not inoculated.

It was found necessary to aerate rather vigorously, otherwise flocculation of the suspended bacteria appeared to take place, resulting in almost complete cessation of growth after 24 to 48 hours.

Two strains were studied sufficiently to give a general idea of the results that may be expected by such methods. The actual quantities of nitrogen fixed by different strains, however, were found in preliminary tests to vary all the way from those here recorded to normal. This was as expected, since any such alteration in nitrogen-fixing ability as here indicated might be expected to vary quantitatively both with the strain and with the period of preliminary culturing upon a high nitrate medium.

From the data presented in tables 4 and 5 it is evident that the 0 culture of both strains 5b and O–2 were active in metabolizing free nitrogen when grown in a suitable liquid medium, the latter fixing somewhat larger quantities than the former.

The 4,000 p. p. m. cultures were incapable of metabolizing more than traces of nitrogen during an incubation period of 4 to 5 days when grown in a nitrogen-free liquid medium—the largest quantity recorded for either culture being 0.12 mg as compared with from 2.91

[7] HUNTER, O. W. STIMULATING THE GROWTH OF AZOTOBACTER BY AERATION. Jour. Agr. Research 23: 665-677, illus. 1923.

to 7.05 mg for the 0 cultures under identical conditions. In only one instance in the 8 experiments of 4 to 5 days' duration could the fixation be regarded as definitely significant, and in this case the actual quantity fixed was only 0.12 mg by the 4,000 p. p. m. culture of strain 5b.

TABLE 4.—*Growth and nitrogen fixation by culture 5b in liquid media varying in nitrogen content*

Experiment No., incubation period and calculated nitrate nitrogen added to media (p. p. m.)	Controls		4,000 p. p. m. culture [1]				0 culture [2]			
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth	Total organic nitrogen per 100 cc media after growth	Nitrogen fixed per 100 cc of media	*P*	Observed growth	Total organic nitrogen per 100 cc media after growth	Nitrogen fixed per 100 cc of media	*P*
	Mg	*Mg*		*Mg*	*Mg*			*Mg*	*Mg*	
Experiment 1; incubation period 4 days:										
0	0	0.15	[3] 0	0.21	0.06	0.3	4	4.31	4.16	0.01
0	0	.15	0	.18	.03	.6				
5	.49	.64	2	.89	.25	.05				
10	1.01	1.16	4	2.03	.87	.01				
20	2.17	2.32	4	3.50	1.18	.01				
Experiment 2; incubation period 4 days:										
0	0	.15	0	.20	.05	.1	4	3.06	2.91	.01
10	1.11	1.26	3	3.03	1.77	.01				
15	1.41	1.56	4	3.67	2.11	.01				
20	2.03	2.18	4	3.70	1.52	.01				
25	2.57	2.72	4	4.21	1.49	.01				
50	5.13	5.28	4	5.94	.66	.01				
75	7.54	7.69	4	7.92	.23	.3				
100	9.97	10.12	4	10.34	.22	.4				
125	12.77	12.92	4	12.77	−.15					
Experiment 3; incubation period 4 days:										
0	0	.09	0	.13	.04	.05				
2½	.25	.34	2	.52	.18	.01				
5	.50	.59	3	1.40	.81	.01				
7½	.75	.84	3	1.40	.56	.01				
10	1.00	1.09	3	1.40	.31	.02				
12½	1.25	1.34	3	1.52	.18	.04				
15	1.50	1.59	3	1.83	.24	.03				
17½	1.75	1.84	3	2.10	.26	.01				
Experiment 4; incubation period 5 days:										
0	0	.09	0	.15	.06	.2	4	5.38	5.29	.01
2½	.25	.34	4	1.86	1.62	.01				
5	.50	.59	4	2.23	1.64	.01				
7½	.75	.84	4	2.63	1.79	.01				
10	1.00	1.09	4	2.93	1.84	.01				
12½	1.25	1.34	4	3.07	1.73	.01				
15	1.50	1.59	4	2.23	.64	.01				
Experiment 5; incubation period 5 days:										
0	0	.09	?	.21	.12	.01	4	4.85	4.76	.01
2½	.25	.34	3	1.28	.94	.01				
5	.50	.59	4	2.11	1.52	.01				
7½	.75	.84	4	2.40	1.56	.01				
10	1.00	1.09	4	1.72	.63	.01				
12½	1.25	1.34	4	2.10	.66	.01				
15	1.50	1.59	4	2.29	.45	.01				
Experiment 6; incubation period 8 days:										
0	0	.09	1	1.09	1.00	.01	4	5.80	5.71	.01
2½	.25	.34	4	3.20	2.86	.01				
5	.30	.59	4	3.00	2.41	.01				
7½	.75	.84	4	4.06	3.22	.01				
10	1.00	1.09	4	3.44	2.35	.01				
12½	1.25	1.34	4	4.27	2.93	.01				
15	1.50	1.59	4	4.67	2.83	.01				

[1] Previously grown at 4,000 p. p. m. of nitrate nitrogen. See text for meaning of *P*.
[2] Previously grown on "nitrogen-free" agar. [3] See footnote 2, table 1.

If the incubation period was lengthened to 8 days, culture 4,000 p. p. m. 5b fixed definite quantities of nitrogen in a nitrogen-free liquid medium, though still much smaller than the 0 5b culture fixed in 4 days. Culture 4,000 p. p. m. O–2 failed to fix significant quantities of nitrogen in a nitrogen-free medium even during an 8-day incubation period.

TABLE 5.—*Growth and nitrogen fixation by culture O–2 in liquid media varying in nitrogen content, with incubation periods of 4 and 8 days*

Experiment No. and calculated nitrate nitrogen added (p. p. m.)	Controls		4,000 p. p. m. culture [1]							
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth after—		Total organic nitrogen per 100 cc of media after growth for—		Nitrogen fixed per 100 cc of media			
			4 or 5 days	8 days	4 or 5 days	8 days	4 or 5 days	*P*	8 days	*P*
Experiment 1:	*Mg*	*Mg*			*Mg*	*Mg*	*Mg*		*Mg*	
0	0	0.09	[3] ?	?	0.12	0.09	0.03	0.2	0	
2½	.25	.34	1	1	.32	.47	−.02		.13	0.02
7½	.75	.84	2	2	1.01	.89	.17	.02	.05	.3
Experiment 2:										
0	0	.09	?	?	.12	.10	.03	.3	.01	.8
2½	.25	.34	1	1	.33	.25	−.01		−.09	
7½	.75	.84	2	2	.94	.87	.10	.05	.03	.2
Experiment 3:										
0	0	.09	?	?	.09	.09	0		0	
2½	.25	.34	?	?	.09	.09	−.25		−.25	
7½	.75	.84	1	1	.09	.36	−.75		−.48	

Experiment No. and calculated nitrate nitrogen added (p. p. m.)	Controls		4,000 p. p. m. culture [2]							
	Nitrate nitrogen per 100 cc media as determined	Total nitrogen per 100 cc media as determined	Observed growth after—		Total organic nitrogen per 100 cc of media after growth for—		Nitrogen fixed per 100 cc of media			
			4 or 5 days	8 days	4 or 5 days	8 days	4 or 5 days	*P*	8 days	*P*
Experiment 1:	*Mg*	*Mg*			*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
0	0	0.09	4	4	7.14	7.26	7.05	0.01	7.17	0.01
2½	.25	.34								
7½	.75	.84								
Experiment 2:										
0	.00	.09	4	4	6.45	6.60	6.36	.01	6.51	.01
2½	.25	.34								
7½	.75	.84								
Experiment 3:										
0	0	.09	4	4	5.73	6.37	5.64	.01	6.28	.01
2½	.25	.34								
7½	.75	.84								

[1] Previously grown as 4,000 p. p. m. nitrate nitrogen. See text for meaning of *P*.
[2] Previously grown on "nitrogen-free" agar.
[3] See footnote 2, table 1.

When the liquid medium contained fixed nitrogen in the form of potassium nitrate, the 4,000 p. p. m. cultures of both 5b and O–2 grew readily and transformed the nitrate nitrogen into organic nitrogen. The quantity of free nitrogen metabolized by the 0 5b culture was small compared with the quantity of combined nitrogen metabolized by the 4,000 p. p. m. 5b culture.

The presence of very small (2½ p. p. m.) quantities of nitrate nitrogen in the medium not only made it possible for the 4,000 p. p. m. culture of strain 5b to grow but also enabled it to readily metabolize free atmospheric nitrogen. Increasing the quantity of combined nitrogen, within a limited range, i. e., up to 5 to 20 p. p. m., increased the ability of this culture to utilize atmospheric nitrogen in every experiment. Further increases, however, resulted in decreased fixation until fixation reached zero, when an adequate quantity was present to supply the needs of the organisms.

In no instance, even when incubation was extended to 8 days, was the 4,000 p. p. m. culture of strain O–2 observed to fix significant quantities of nitrogen when grown in a liquid nitrogen-free medium. Even when small quantities of nitrate nitrogen were added to the medium, fixation was so small and irregular as to suggest that some other factor might have been responsible for the apparent significant increases in nitrogen here recorded.

The almost complete absence of nitrogen fixation in the 4,000 p. p. m. O–2 cultures raised the question of the possibility of the *Azotobacter* having been supplanted by a contaminant so similar morphologically and culturally as to have escaped notice. However, reisolations from liquid cultures upon agar, even though initial growth was very slow, followed by numerous transfers, gradually brought the culture back to where definite fixation of nitrogen could be demonstrated, proving conclusively that the culture was a modified strain of *Azotobacter*.

DISCUSSION AND SUMMARY

It is evident from the data presented that prolonged growth of *Azotobacter* in the presence of relatively high concentrations of potassium nitrate resulted in such an alteration in the physiological make-up of certain strains of the organism as to render them incapable of growing normally in the absence of combined nitrogen. Such a condition was reflected (1) in very limited or no visible growth, (2) inability to metabolize atmospheric nitrogen, or (3) a combination of both conditions. It is believed that the effect of the potassium nitrate was due to the nitrogen since potassium ions supplied in other salts did not have a similar effect. The discussion is restricted for the most part to nitrate nitrogen because the experiments were largely concerned with this form. Limited experimentation with other nitrogenous compounds indicated that they would give similar results provided the nitrogen was available to *Azotobacter*.

All strains that did not grow on the nitrogen-free medium grew when small quantities of potassium nitrate were added to the medium. In the case of certain strains such growth, following previous growth at high concentrations of nitrate nitrogen, was proportional to the added nitrogen, and quantitative data proved the absence of nitrogen fixation. In the case of other strains the presence of very small quantities of nitrate nitrogen sufficed to initiate growth and subsequent growth was more or less independent of the quantity of nitrogen initially present. Quantitative data proved in such instances that atmospheric nitrogen was readily utilized. In the former case the organisms apparently lost, at least temporarily, their ability to fix free nitrogen, whereas in the latter case this ability was conditioned

upon growth and was readily regained when favorable growth conditions were reestablished. In other words, very limited quantities of combined nitrogen served to restore some, but not all, strains to normal within a very short time.

Just what alteration occurred in the physiological make-up of those strains that underwent the suggested change is not known. The available evidence indicates temporary or more or less permanent loss of the ability to metabolize atmospheric nitrogen. As a possible explanation of the change it might be assumed that when grown under conditions where nitrogen fixation is completely suppressed for an adequate length of time, the organisms cease to elaborate the nitrogen-fixing enzyme system. If the further assumption is made that combined nitrogen is essential for the elaboration of the enzymes necessary in nitrogen fixation it can be easily understood why the presence of limited quantities of nitrate nitrogen restored the free-nitrogen metabolizing ability in those strains regaining normalcy in its presence. It is also not inconceivable that even the ability to elaborate the enzyme system was lost by those strains that failed to return to normal in the presence of low concentrations of nitrate nitrogen.

THE REACTIONS OF WIREWORMS TO ARSENICALS[1]

By CHARLES E. WOODWORTH[2]

Associate entomologist, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

It has been noted (*4, 7*)[3] that arsenicals in bran or whole-wheat baits render these baits relatively unattractive to wireworms. It has also been noted that the mortality of wireworms captured by this method is very little more than when unpoisoned baits are used. Since arsenic is generally very toxic to insects, these observations have aroused considerable speculation. The present investigation was designed to explain the failure of baits poisoned with arsenic to attract and kill wireworms, and to obtain a better understanding of the mode of entry of arsenic into the body of the larva. The Pacific coast wireworm (*Limonius canus* Lec.) was selected for this study, as it is one of the four most important species, from an economic standpoint, in the irrigated lands of the Pacific Northwest. The other three species, *L. californicus* (Mann.), *L. subauratus* Lec., and *L. infuscatus* Mots., belonging as they do to the same genus, differ only slightly in morphology and physiology from the species selected; so the results are for the most part applicable to all four species.

EXPERIMENTAL PROCEDURE

TESTS WITH CORNSTARCH PELLETS CONTAINING ARSENICALS

In order to study the effects of arsenic upon wireworms, larvae were confined in glass cages with cornstarch pellets containing an arsenical. The pellets were made of 4 parts of cornstarch and 1 part of the arsenical, moistened with sufficient distilled water to prevent crumbling. Cornstarch was used because it is an attractive food for this species and does not get hard after it is wet, as does wheat starch. The arsenicals used were acid lead arsenate, paris green, mercurous arsenite, acid sodium arsenate, and sodium arsenite. Unpoisoned cornstarch pellets were used as checks. Cornstarch made alkaline to pH 10 with sodium hydroxide was also used as a check to determine whether the alkalinity of sodium arsenate and sodium arsenite had any effect on their repellency. The other arsenicals added to the cornstarch were either acid or neutral. Fast green to a concentration of about 0.01 percent was sometimes added to the mixtures, as this material could be seen through the body wall and was found to be nontoxic. The mixtures were cast in $\frac{3}{16}$-inch holes in a piece of sheet iron three thirty-seconds of an inch thick. The pellets were pushed out with a sawed-off nail and allowed to dry in air. For these studies 256 larvae were used, including the checks, and were kept

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[2] The author is indebted to M. C. Lane, in charge of wireworm investigations in the Pacific Northwest, Walla Walla, Wash., under whose direction the work was conducted.

[3] Italic numbers in parentheses refer to Literature Cited, p. 237.

individually in the cages with the pellets. All the dead wireworms and an adequate sample of the live ones from each group were tested for arsenic.

TESTS WITH CORNSTARCH PELLETS CONTAINING NONARSENICALS

Several nonarsenicals, both liquids and solids, were also incorporated in cornstarch pellets and placed in glass cages with the wireworms to determine whether the materials were ingested, and, if so, in what form. Finely ground graphite or lampblack, both inert substances, was mixed in the ratio of 1 part to 10 parts of cornstarch. The former was of a particle size that was easily recognizable under the microscope, and the latter colored the gastric content when taken in sufficient quantity. Iodine at a concentration of 0.1 percent and neutral red and Sudan III at a concentration of 0.05 percent were also used. Iodine stains starch a deep blue black and gives the grains their characteristic markings. Neutral red is a nontoxic dye. Sudan III is a fat-specific dye which gives a pink color to fats. Any of these stains should therefore be easily recognizable in the gastric content. Examination for these materials was made under a binocular microscope during the dissection of the wireworms and under the compound microscope in the study of the digestive-tract content. Starch was searched for by the familiar iodine test (*2, p. 93*) and reducing sugar by the Benedict test (*2, p. 744*). A total of 91 wireworms was used in these tests.

The cages that were used for the tests consisted of two microscope slides held apart by channeled metal supports and filled with a pad of moist cellucotton.[4] A cornstarch pellet was placed in one corner of each cage. The humidity of the atmosphere in the cages was maintained at about 100 percent by moistening the cellucotton pad and keeping the cages in a desiccator jar containing a little distilled water. The temperature was kept constant at 68° F. The period of confinement varied, but was usually more than 96 hours and occasionally as long as 1 month.

TESTS FOR ARSENIC

Tests for arsenic were made in a modified Gutzeit apparatus (*1, p. 371*), in which the reaction chamber was a 12- by 35-mm shell vial and the rest of the apparatus was reduced proportionately. The scrubber was a piece of 12-mm glass tubing 5 cm long and narrowed to 6 mm at the lower end, filled with loosely packed absorbent cotton that had been soaked in saturated lead acetate and squeezed free of excess liquid. The test papers were cut 2 by 100 mm, soaked in 4-percent alcoholic mercuric bromide solution for 30 minutes, and dried in air for about 20 minutes immediately before being used. The prepared paper strip was held in an 8-cm length of 4-mm glass tubing. All connections were made with the lower half of No. 00 rubber stoppers. The sensitivity was found to be about 1 microgram of arsenic. Blanks and checks of either 0.05 or 0.10 cc of 0.001 *N* sodium arsenite were run with each test. The checks were compared with a set of standards before the results were recorded.

Because of the proteinaceous and fatty nature of the insect material, an alkaline digestion was used rather than the acid one usually recommended. The procedure was as follows: The dissected material and

[4] WOODWORTH, C. E. A METAL AND GLASS INSECT CAGE. U. S. Bur. Ent. and Plant Quarantine, Multigraphed Cir. ET-69, 3 pp., illus. 1935.

five drops of 15-percent sodium hydroxide were placed in the shell-vial reaction chambers and held in a water bath for 30 minutes at a temperature just below the boiling point. Five drops of concentrated hydrochloric acid were then added. The mixture was allowed to stand 10 minutes at room temperature. Two drops each of 15-percent potassium iodide and 40-percent stannous chloride solution made up with concentrated hydrochloric acid were then added. The concoction was allowed to stand for another 10 minutes, after which about 0.33 g of zinc was added and the rest of the apparatus, which had been assembled, was immediately put in place. Precautions were taken to use arsenic-free reagents throughout the experiment.

TESTS WITH ARSENICAL SOLUTIONS

Experiments were also conducted in which the wireworms were submerged in solutions of sodium arsenite and acid lead arsenate and in distilled water. The concentrations of sodium arsenite that were used were 0.25, 0.5, 1, and 2 percent. For lead arsenate a saturated solution was used, which was found to contain about 0.75 microgram of arsenic per cubic centimeter. These solutions were put in 12- by 60-mm shell vials, 2.5 cc per vial. One wireworm was submerged in each vial, and groups of 10 were kept for different periods of time at a temperature of 68° F. They were then tested for gain in weight and survival or dissected and tested for arsenic. The lead arsenate was used only in preliminary survival tests.

In the experiment to determine the gain in weight during submergence 350 wireworms were used. These were divided into 7 groups of 10 each for each of the 4 concentrations of sodium arsenite and for the distilled water. All larvae were weighed before submergence, and those of group 1 at the end of 3, 6, 12, 24, 48, 96, and 192 hours. The other six groups were weighed only once or twice after submergence, to offset the effects of repeated handling. The two groups that were weighed twice were weighed at 6 and 192 hours and at 12 and 384 hours. All the wireworms in the last six groups were set aside after their final weighing to be tested for survival.

In the dissection tests wireworms were submerged in a 2-percent sodium arsenite solution for various periods of time. They were then washed, the blood taken, and dissected. The various tissues were tested for arsenic. The blood was obtained by piercing the integument, usually in the pleura of the prothorax, and then pressing the abdomen slightly until the blood came through the hole and stood out as a drop. The blood was readily removed by use of a capillary tube and transferred to the Gutzeit apparatus for testing. The question arose as to whether the blood might not get its arsenic from the integument. To answer this question a drop of distilled water was used to wash the larva just before the blood was taken, and this water was tested for arsenic. In all, 135 tests were made, though not all tissues were tested each time. Frequently just the blood and the digestive tracts were tested, and all the earlier tests were made without testing the wash water. Each test was made with only one wireworm.

RESULTS

TESTS WITH CORNSTARCH PELLETS CONTAINING ARSENICALS

The results of the tests with cornstarch pellets containing arsenic are shown in table 1. At first it appeared that it would be very easy

to obtain the median lethal dose, as the wireworms usually burrowed into the poisoned pellets and apparently fed. They quickly riddled the cellucotton pads with their burrowing and at times molted in the cages. In all but the sodium arsenite and sodium arsenate groups a large proportion of the pellets were riddled. The repellency was about 10 percent for the unpoisoned pellets, 24 percent for the lead arsenate groups, 31 percent for the mercurous arsenite groups, 37 percent for the paris green groups, 75 percent for the sodium arsenite groups, and 82 percent for the sodium arsenate groups. The alkalinity of the sodium salts of arsenic was not the cause of their high repellency, since the larvae that were placed with sodium hydroxide (pH 10) exhibited only a 30-percent repellency.

In all cases where fast green was used, the dye spread by capillary action from the pellet into the cellucotton, in which the wireworms moved. Tests for arsenic on portions of some of the pads from the sodium arsenite cages showed that the arsenical also had spread into the pads. In one case it had reached a concentration of 0.05 mg per square centimeter of pad at the far end of the cage.

In the sodium arsenate and sodium arsenite groups the attack was very slight; only 2 of the 19 wireworms that attacked the pellets were dead, and these contained arsenic in the digestive tract but not in the blood. In all but one case where arsenic was found in the blood the quantity was large. It was also found in these wireworms in readily measurable quantities in the muscle and fat, but was entirely lacking in the digestive tract. In the one case where arsenic was found in the blood of a living wireworm the quantity was minimal and barely recognizable. Similar sublethal quantities were found in the digestive tract of four live individuals.

TABLE 1.—*Results obtained in tests on wireworms confined with arsenical pellets*

Material added to cornstarch	Wireworms used	Pellets attacked	Wireworms showing fast green in digestive tract	Live wireworms not tested for arsenic [1]	Wireworms tested					
					No arsenic found		Arsenic found			
							In digestive tract		In blood	
					Alive	Dead	Alive	Dead	Alive	Dead
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Lead arsenate	29	20		17	9	1	0	1	1	0
Lead arsenate and fast green	20	17	6	10	10	0	0	0	0	0
Paris green	25	15		13	12	0	0	0	0	0
Paris green and fast green	18	12	1	8	9	1	0	0	0	0
Mercurous arsenite	8	6		2	5	0	1	0	0	0
Mercurous arsenite and fast green	8	5	1	4	3	0	1	0	0	0
Sodium arsenate	25	4		10	7	0	0	1	0	7
Sodium arsenate and fast green	19	4	1	9	8	0	1	0	0	1
Sodium arsenite	25	6		7	13	0	0	1	0	4
Sodium arsenite and fast green	19	5	1	9	4	0	1	0	0	5
Checks:										
Cornstarch alone	25	23		13	10	2	0	0	0	0
Fast green	25	22	12	15	9	1	0	0	0	0
Sodium hydroxide (pH 10)	10	7		9	0	1	0	0	0	0
Total	256	146	22	126	99	6	4	3	1	17

[1] All dead wireworms were tested for arsenic.

TESTS WITH CORNSTARCH PELLETS CONTAINING NONARSENICALS

The results of the tests with nonarsenical pellets are shown in table 2. Of the 25 wireworms that received cornstarch alone, the 9 that are recorded as giving negative results either failed to feed or gave negative tests for starch when the content of the midintestine was studied. The iodine test did not reveal starch in the digestive tract even though the pellets showed clearly that a portion of the starch had been removed. The sugar test failed to demonstrate the presence of sugar in the digestive-tract content, though this might be accounted for by the small quantity of material available for the tests. The 16 doubtful cases were those individuals that were used as checks in other experiments and were not tested specifically for starch.

Of the 25 wireworms that were given access to the cornstarch and fast green, the 9 reported as doubtful were those that were not dissected and in which fast green was not visible through the integument, though the pellets had been attacked. Fast green is a hydrogen-ion indicator, the range being comparable with that of methyl red. There was no indication of an alkaline reaction in any part of the digestive tract. The doubtful cases with lampblack, neutral red, and Sudan III were those in which the color in the digestive tract was not sufficiently intense. Sudan III colored the fat bodies of all wireworms confined with this material, but was not definitely recognizable in the digestive tract in any case. The two doubtful cases with iodine were those in which a brown coloration was noted in the tract but it was not definitely caused by the iodine. This material appears to be repellent to wireworms.

TABLE 2.—*Results of tests on wireworms confined with nonarsenical pellets*

Material added to cornstarch	Wireworms used	Pellets attacked	Dead wireworms	Wireworms in which material was recognizable in digestive tract		
				Positive	Doubtful	Negative
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
None	25	23	2	0	16	9
Fast green	25	22	1	12	9	4
Graphite	10	6	0	0	0	10
Lampblack	12	9	1	5	3	4
Iodine	8	4	1	0	2	6
Neutral red	7	4	0	2	1	4
Sudan III	4	4	0	0	2	2

TESTS WITH ARSENICAL SOLUTIONS

It was noted in the experiments with the pellets that arsenic entered the blood but not the digestive tracts of wireworms that were on saturated cellucotton pads in which arsenic was found to be present. In preliminary trials submerged wireworms gained weight very rapidly, some larvae more than doubling their weight in 2 weeks. Table 3 gives the results of some experiments designed to determine the rate of increase in weight of larvae after various periods of submergence in sodium arsenite solution. The wireworms soon became bloated and turgid, and after 4 days certain individuals were hard to handle because of the danger of causing a rupture. As they were weighed in

groups of 10, a single ruptured individual would cause a considerable drop in the average weight. Dissection of some of the bloated wireworms showed that the digestive tract was slightly swollen, but not in proportion to the rest of the body. Mortality studies showed that the rate of killing was proportional to the time of exposure and the concentration of sodium arsenite. Saturated solutions of lead arsenate did not kill 100 percent of the wireworms even after submergence for a week. The mortality of the larvae in this material was comparable with that in distilled water for the same period.

TABLE 3.—*Rate of increase in weight of wireworms submerged in sodium arsenite solutions*

Concentration of sodium arsenite	Average original weight of wireworms	Average gain in weight of wireworms after submergence for—							
		3 hours	6 hours	12 hours	24 hours	48 hours	96 hours	192 hours	384 hours
Percent	*Milligrams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
[1] 0.00	24.2	14.1	23.3	38.8	58.3	75.1	76.9	77.7	[2] 67.4
.25	24.1	15.7	24.0	35.0	62.6	71.2	74.9	[2] 72.5	[2] 84.7
.50	24.3	11.6	22.5	40.5	65.6	71.8	84.2	85.2	[2] 72.0
1.00	24.3	10.8	20.0	36.3	46.5	69.2	78.8	82.8	88.4
2.00	24.1	9.1	14.0	28.0	45.0	65.8	[2] 62.1	[2] 80.1	[2] 63.8

[1] Distilled water.
[2] 1 or more individuals ruptured.

TABLE 4.—*Quantity of arsenic found in parts of wireworms that had been submerged in 2-percent sodium arsenite solution*

Part tested	Arsenic found [1] after submergence for—						
	3 hours	18 hours	24 hours	48 hours	96 hours	144 hours	192 hours
Wash water	None	[2]	Trace	Trace	None	[2]	Trace.
Blood	Little	Much	Much	Much	Much [3]	Much [3]	Much.[3]
Fat	Trace	Little	Little	Little	do	Little	Do.
Digestive tract	do	None	None	None	Trace	Trace	Trace.
Nerve	do	do	do	Trace	None	None	None.
Heart	None	do	Trace	do	Trace	do	Do.
Tracheae	do	do	None	None	None	do	Do.
Muscle	Trace	Trace	Little	Little	Little	Little	Much.
Integument	do	[2]	do	do	do	do	Little.

[1] "Trace indicates 2 micrograms or less; "little," more than 2 micrograms and less than 10 micrograms; "much," 10 or more micrograms.
[2] Not tested.
[3] More than could be recorded on test paper.

Since the increase in weight of the submerged wireworms indicated that liquids do permeate the body wall, the next problem was to find out whether the arsenical salts are carried through with the liquids. Tests for arsenic in various parts of individual wireworms that had been submerged are reported in table 4. In this table it is shown that the quantity of arsenic in the blood increases with the increase in total weight as recorded in table 3. The wash water, on the other hand, does not show any such consistent increase. When the larvae were taken out of the solution, rinsed, and placed on a moist blotter, there was an increase in the quantity of arsenic that could be washed off the integument as time elapsed, some tests showing as much as 5 micrograms. The quantity of arsenic in the fat bodies varied in general

with the quantity of fat and with the length of exposure. The digestive tract, the nerve cord, and the heart showed practically no arsenic, but in the case of the last two this may have been due in part to their small size. The tracheae were consistently free of arsenic. Both the muscles and the integument showed relatively large quantities of arsenic. The muscles usually contained more arsenic than the integument.

DISCUSSION

Contrary to expectation, in the tests with pellets containing arsenic those wireworms that apparently fed did not die. It was noted that certain of the poisoned pellets were markedly repellent to the wireworms. This repellency was found to be more or less in proportion to the solubility of the arsenical present. Nevertheless, the majority of the wireworms that were killed were in the cages with the highly repellent and highly soluble sodium arsenate or sodium arsenite. It was further found that the majority of those that were killed had not attacked the poisoned pellets, and that arsenic was not present in their digestive tracts. In only three cases, or about 1.5 percent of the tests, was arsenic found in the alimentary canal in quantities sufficient to kill. In four other cases the poison was found there, but not in fatal quantities, and the wireworms were still active and appeared normal until they were killed for examination.

Wireworms were repeatedly noted burrowing into arsenically poisoned pellets, with no apparent ill effects. This led to the question as to whether solids are ever eaten by wireworms. Langenbuch (*3*) decided that it was necessary for foods to be in solution before they would be ingested. Repeated observations at this laboratory showed that soil particles were never recognizable in the gastric content, which fact seems to support that view. Lampblack, however, was found in the digestive tracts of several wireworms that had burrowed into pellets containing that material. This showed that solids may be ingested if the particle is small enough. Fine graphite, on the other hand, has a particle size a little larger than that of lampblack, and it was rejected in every case where it was tried.

The Pacific coast wireworm possesses a mechanical means of preventing undesirable substances from entering the digestive tract. In the anterior part of the buccal cavity there are two sclerotic plates which fit tightly together when relaxed, closing off the digestive tube completely. These plates can be separated only by contractions of large muscles which are attached to them and which are controlled by the central nervous system. In addition, there are more than a thousand bristlelike hairs in the preoral cavity, distributed over the anterior edge of the ventral closing plate, on the hypopharynx, and on the mandibles, maxillae, and labial palpi, which act as a filtering device to keep large particles from reaching the digestive tube. This apparatus is probably sufficient to prevent even undesirable soluble materials from entering the digestive tract. The fact that dyes and other nonpoisonous materials were taken in indicates that the larvae have sensoria and can discriminate against the arsenicals. It is concluded, therefore, that the reason arsenically poisoned baits do not kill wireworms is that arsenicals are repellent to them and the larvae do not ingest the poison even when they do come to the baits.

Cornstarch, which has a larger particle size than graphite, was

never recognized in the gastric content. Corn as well as wheat is considered a desirable food for wireworms, frequently being used in rearing experiments. In such experiments the wireworms are commonly found cleaning the starch completely from the seed coat, leaving no pile of rejected refuse. It appears, then, that either very fine grinding or predigestion must take place. The failure of the iodine test to show its characteristic blue color when applied to the gastric content indicates that predigestion must take place and that the starch is reduced at least to achroödextrin before it reaches the stomach. Iodine-stained starch was burrowed into, but the gastric content failed to show either the starch granules or the typical starch-iodine color, which also indicated that predigestion had taken place if the starch was eaten. The tests for reducing sugar were inconclusive. The failure to obtain a positive test did not necessarily mean that digestion beyond achroödextrin had not taken place. The quantity of material for each test was so small that the reduced copper could easily have been overlooked.

Fast green was frequently found in the bodies of wireworms that had access to pellets containing the dye. At times it was found in the wireworms even though the pellets had not been attacked. The capillary action of the fast green through the cellucotton pads gave the suggestion that soluble arsenicals might also move through the pads. Tests showed this to be true, and thus it was seen that sodium arsenite and sodium arsenate were brought in contact with the body of the wireworm even though the pellets were not attacked.

By far the greatest number of wireworms that died in the cages with the poisoned pellets did not have so much as a trace of arsenic in their digestive tracts. Most of them did, however, have large quantities of arsenic in the blood. The arsenic must therefore have entered the body by some means other than by way of the mouth. This finding is contrary to that of Subklew (*9*), who states that salts must enter the body through the digestive tract.

When the wireworms were submerged in arsenical solutions, they took on weight rather rapidly. In the first 6 hours this increase was between 20 and 24 percent, except in the hypertonic 2-percent solution. Tests for arsenic showed that the quantity of arsenic in the blood increased according to the time of submergence. The arsenic in the digestive tract did not increase noticeably, and the concentration of arsenic in the haemocele was constantly and increasingly greater than in the gastric content, showing conclusively that the arsenic did not enter by either the mouth or the anus.

These facts leave only two possible ways in which the arsenic may enter the body, through the tracheae or through the integument. The former is eliminated in the finding of a complete lack of arsenic in the tracheae, and by the observation that these organs were filled with air in all cases where dissections were made. These tubes filled with liquid only after they had been cut and the gases allowed to escape. The integument, then, is the only tissue through which the arsenic could have passed. This is contrary to the generally accepted idea, recently reiterated by Wigglesworth (*10*, *p. 2*), that the integument is impermeable. Mellanby (*6*), too, in his study on site of loss of water from insects, concludes that all the integument is watertight.

Subklew (*9*) admitted that water might pass through the integument but denied that salts could pass through that tissue. The present study shows definitely that at least the sodium salts of arsenic, when in solution, can and do pass through the integument of the wireworm. The finding of arsenic in the body wall supports this conclusion, as does the recent work of O'Kane and Glover (*8*) and Lepesme (*5*).

SUMMARY

This paper reports a series of experiments designed to explain the failure of baits poisoned with arsenicals to attract and kill wireworms. Experiments are also presented that show how and where soluble arsenical salts in solution may enter the body of the wireworm.

In the early experiments the wireworms were confined in metal and glass cages, on moist cellucotton pads with cornstarch pellets. These pellets were made by mixing various arsenicals or nontoxic materials with cornstarch, either with or without the addition of dyes, and casting the moistened mass. The test for arsenic was by the Gutzeit method, but in a modified apparatus reduced to the size that samples of one drop or less could be tested, and an alkaline digestion was used.

Arsenicals were found to be repellent to wireworms more or less in proportion to their solubility. The majority of wireworms that were killed in these experiments were confined with the more repellent materials. Furthermore, most of the wireworms that died had no arsenic in the digestive tract, but had large quantities in the blood. Very firm, solid substances were demonstrated as being ingested, and certain soluble ones were taken in. Arsenicals were rejected, even though the pellets containing them were burrowed into. The sensory control of the closing mechanism in the buccal cavity may account for the rejection of the arsenic.

In the latter part of the study the wireworms were submerged in arsenical solutions and the various tissues tested for the presence of arsenic. Water containing sodium arsenite entered the bodies of the wireworms, carrying the arsenic with it. It did not enter by way of the mouth, anus, or tracheae, but through the integument, which was found to be pervious both to water and to the sodium salts of arsenic.

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